PUERTO RICO EXPERIMENT STATION

of the

UNITED STATES DEPARTMENT OF AGRICULTURE

MAYAGUEZ, PUERTO RICO

REPORT OF THE PUERTO RICO EXPERIMENT STATION

1943

Issued — 1944

UNITED STATES DEPARTMENT OF AGRICULTURE

AGRICULTURAL RESEARCH ADMINISTRATION

OFFICE OF EXPERIMENT STATIONS

For sale by the Superintendent of Documents, U. S. Government Printing Office
Washington 25, D. C. - Price 10 cents

PUERTO RICO EXPERIMENT STATION

Administered by the Office of Experiment Stations
Agricultural Research Administration
United States Department of Agriculture

JAMES T. JARDINE, *Chief, Office of Experiment Stations*

STATION STAFF

KENNETH A. BARTLETT, *Director.*
MERRIAM A. JONES, *Associate Chemist.*
HAROLD K. PLANK, *Associate Entomologist.*
MILTON COBIN, *Associate Horticulturist.*
CLAUD L. HORN, *Associate Horticulturist.*
ROY E. HARPER, *Associate Plant Geneticist.*
RUFUS H. MOORE, *Associate Plant Physiologist.*
BARTON C. REYNOLDS, *Assistant Agricultural Engineer.*
JOSÉ O. CARRERO, *Assistant Chemist.*
HOWARD T. LOVE, *Assistant Chemist.*
DAVID L. STODDARD, *Assistant Plant Pathologist and Physiologist.*
ARMANDO ARROYO, *Junior Scientific Aide.*
CARMELO ALEMAR, *Junior Administrative Assistant.*
JUANA F. CEDÓ, *Assistant Clerk-Stenographer.*
DALTON W. MILLER, *Property Clerk.*
CARMELO ALEMAR, Jr., *Collaborating Agronomist.*[1]
JACINTO RIVERA PÉREZ, *Collaborating Agronomist.*[1]
ERNESTO HERNÁNDEZ MEDINA, *Collaborating Agronomist.*[1]
JOSÉ E. NATAL COLÓN, *Cooperating Agronomist.*[1]
FRANCISCA E. ARANA, *Collaborating Chemist.*[1]
NOEMÍ G. ARRILLAGA, *Collaborating Chemist.*[1]
RAFAEL FANTAUZI, *Collaborating Agricultural Engineer.*[1]
EFRAÍN AVILÉS LOJO, *Cooperating Assistant Clerk.*[1]
ASTOR GONZÁLES, *Cooperating Librarian.*[1]
VIOLETA VICENTE, *Cooperating Assistant Librarian.*[1]

[1] In cooperation with the Government of Puerto Rico.

PUERTO RICO EXPERIMENT STATION
of the
UNITED STATES DEPARTMENT OF AGRICULTURE
MAYAGUEZ, PUERTO RICO

Washington, D. C. 1944

REPORT OF THE PUERTO RICO EXPERIMENT STATION, 1943

CONTENTS

INTRODUCTION

Wartime conditions have brought about scarcities of many strategic agricultural materials, such as the insecticide rotenone, which is obtained from various plants. For many years the Puerto Rico Experiment Station has assembled a collection of tropical plants from all over the world, including high-yielding strains of *Derris elliptica* (Roxb.) Benth., the chief source of rotenone. As a result, the station was in the unique position of having available the only large source of propagating material of this plant in the Western Hemisphere. The rapid and early establishment of extensive plantings from this source in Latin America will thus alleviate the scarcity of rotenone in the shortest possible time. During the course of the fiscal year 634,310 cuttings were distributed to 11 different foreign countries and Hawaii.

Other station activities of importance to the war effort included the providing of large quantities of seed for use in the local food-production program. Likewise, thousands of plants and seeds have been distributed to the military forces for use in the camouflage of new military bases throughout the island.

The stock of high-yielding strains of *Cinchona*, from which quinine is obtained, was expanded, and a new area for permanent plantings was opened in the Toro Negro Forest area.

PERSONNEL

Atherton Lee, director of the station since 1934, was on loan to the Office of the Coordinator of Inter-American Affairs, beginning November 1941. On February 2, 1942, he was transferred to that Office at their request. Kenneth A. Bartlett, who had been serving as acting director, was appointed director, effective December 16, 1942.

Arthur G. Kevorkian, assistant plant pathologist and physiologist, resigned, effective July 1, 1942, and was succeeded by David L. Stoddard, who reported for duty December 1, 1942.

Claud L. Horn, who had been working with the station since 1935 as associate horticulturist in charge of plant introductions, resigned December 15, 1942, to join the Office of Foreign Agricultural Relations, and was replaced by Milton Cobin.

The station suffered a great loss in the death of Armando Arroyo, scientific aide, who had been connected with the station for over 24 years. Mr. Arroyo died in line of duty November 24, 1942.

Two members of the staff, Howard T. Love, assistant chemist, and Dalton W. Miller, property clerk, joined the armed forces June 28, 1943.

Minnie C. Burgess, principal stenographer, resigned January 31, 1943, and was replaced by Juana F. Cedó, assistant clerk-stenographer.

A number of changes took place during the year in the Insular Government personnel who cooperate with the station. José E. Natal Colón resigned August 31 as agronomist on the essential-oil project and was replaced by Jacinto Rivera Pérez, who was transferred from the position of agricultural engineer on the bamboo project. Rafael Fantauzi was appointed as engineer on the bamboo project September 10, and since his resignation, on February 28, this position has remained vacant. The vanilla agronomist, Ernesto Hernández Medina, resigned April 10; the duties which he had been performing were supervised from then to the close of the fiscal year by Jacinto Rivera Pérez. The vanilla chemist, Francisca E. Arana, resigned June 2; the chemistry phase of the vanilla program has been discontinued.

Rufus H. Moore served as acting director for a period of about 1 month during the absence of the director at the second Inter-American Conference of Agriculture, held at Mexico City.

Harold K. Plank served as acting director during a period of about 2 months during the absence of the director in Washington.

COOPERATION WITH OTHER GOVERNMENT AGENCIES

As in past years, the station has continued to receive whole-hearted cooperation from all other governmental agencies in Puerto Rico, both Federal and Insular. The Insular Government appropriated $26,900 to carry on cooperative studies of vanilla, spices, essential oils, and the utilization of bamboo. Through its War Emergency Program, the Insular Government also provided relief labor to supplement the above projects as well as Federal projects being carried on at the station.

The Work Projects Administration has continued to make available labor assistance for the expansion of the cinchona and insecticidal-plant programs.

The station has cooperated closely with the Office of Foreign Agricultural Relations and, in conjunction with the programs for the establishment of experiment stations throughout Latin America, that office has sent personnel to Mayaguez to study the activities and projects of the station. In addition, O. F. A. R. assigned an agriculturist to cooperate in the work on the propagation of *Derris* and *Lonchocarpus;* a study of the effect of growth substances is being made in order that as large quantities as possible of such plant material may be available for distribution.

For a period of several months the Office of the Coordinator of Inter-American Affairs assigned two men to work at the station, one on the production of local food crops and the other on the design and construction of bamboo furniture. The latter project was later assumed by the Puerto Rico Development Co. and continued on a cooperative basis, resulting in the establishment on the island of a new furniture industry utilizing bamboo.

The station continued to provide laboratory space, offices, and field areas for the work of the Soil Conservation Service being carried on in the Mayaguez area, and office space for the Farm Security Administration.

Two members of the station staff were on assignment to the Board of Economic Warfare for a period of about 6 months for studies concerned with the distribution of *Derris* and the growing of fiber crops in the Western Hemisphere.

The local food-production office of the W. P. A. was located at the station, as were also those of the Insular inspector of fertilizers and the Insular plant-quarantine inspector collaborating with the Bureau of Entomology and Plant Quarantine.

The experiment station of the University of Puerto Rico was provided with office, laboratory space, and field areas for carrying on experimental work with coffee. In addition, the local staffs of the two stations cooperated on a war project concerned with the effect of growth-promoting substances on the production of vegetables.

The director of the station served as a member of the USDA War Board, and the facilities of the station were utilized in various ways for the carrying out of the programs and policies of the Board.

CINCHONA

Field studies: Roy E. Harper.

Experiments and observations on the light requirements of *Cinchona* seedlings have indicated an interesting relationship between root development and growth and the light needed during the various nursery stages. Seedbed shelters prepared in July and completely enclosed with 10-ounce canvas so that light to the extent of only 50 to 75 foot-candles was admitted during the brightest period of the day gave excellent results. Upon complete germination about 4 weeks after seeding, light was increased at the rate of 25 foot-candles weekly for 3 weeks, and 50 to 60 foot-candles weekly for the next 12 weeks.

These seedlings when transplanted at 4 months of age sustained light injury unless the light was decreased to about one-half that of the original bed. Light tolerance appeared to be associated with root development, and root development was inhibited in nursery beds containing excess moisture. Seedlings under such conditions were more sensitive to light than those in beds in which the moisture was under control and the surface of the beds was allowed to dry out occasionally.

Cinchona seedlings 8 to 12 months old grow slowly if exposed to relatively small quantities of light. Seedlings of this age grew only approximately 1 or 2 inches in 6 months when kept in a maximum light of 150 to 200 foot-candles, whereas growth was approximately 4 inches in light of 400 to 500 foot-candles and approxi-

mately 8 inches in light of 750 foot-candles and above. During this period any direct light seemed to retard growth, the amount of retardation depending upon the amount and duration of direct sunlight admitted.

After *Cinchona* seedlings have been in the seedbed 5 to 7 months, it has been found good practice to transplant them to larger nursery beds. Satisfactory beds were constructed of pole frames built to support a simple roof sloping to the south, 5 feet high in front and 3½ feet at the back. The most satisfactory leaves for shading were found to be those of the "sierra" palm (*Euterpe globosa* Gaertn.).

The clay soils of the Maricao area, where the nursery beds are located, were found to be too heavy for satisfactory seedling growth and have indicated some toxicity. Various media were tried in the nursery beds, using forest duff alone and in various combinations with calcareous sand and a coarse sandy loam. Of these the forest duff alone proved most satisfactory, both in survival and growth. The mixture with calcareous sand gave a good soil structure, but root development was inhibited. The coarse sandy loam did not give satisfactory drainage.

In another trial a siliceous silt loam and a sand of serpentine origin in mixture with forest duff also gave poor results. In a later trial Vivi coarse sand, a river sand of granitic origin, was found somewhat superior to any of the other combinations; 3 months after transplanting, growth was superior and mean survival was 22 percent greater than with other mixtures.

Some damage to the nursery beds has resulted from the activity of earthworms, which rapidly break down the forest duff in the soil mixtures and thus impede drainage and aeration. This damage was greatest in duff alone and in mixtures high in duff. It is also thought that the earthworms may cause toxicity by bringing up enough of the Nipe clay soil from below the bed. This type of damage has been overcome to some extent by building the beds over subsoil. These beds tended to remain uninfested by worms for a much longer period.

An experiment was designed to study the effect on *Cinchona* seedlings of various ecological complexes in the same general locality in the Maricao Insular Forest. At different points in the forest, 12 nursery beds, composed of forest duff and leafmold [1] found in the immediate vicinity, were built upon the forest floor to a height of 12 to 15 inches. Each bed or plot, selected for the best utilization of natural shade, was made more or less rectangular and of a size sufficient to accommodate 42 to 108 young seedlings planted 8 by 8 inches. At the time of transplanting, artificial shade was provided when the shade from surrounding trees was insufficient and later removed when the seedlings had become established. Plant-height measurements were taken about 15 days after transplanting and again about 200 days later. Upon making an arbitrary classification of the environmental factors affecting each plot, it was found that certain plots could be grouped together as having the same combination of major environmental factors. The plots were classified into 7 such groups. Two criteria of comparison were used, namely, seedling survival and mean growth during the 200-day period.

[1] A distinction was made between forest duff, which occurs as spongy, friable, fairly stable humus matted with tree roots over rocky areas, and leafmold, which is a mixture of leaves and twigs in a more active state of decomposition.

The best group consisted of two plots built over rocks in a location in which soil was completely absent. The plots were constructed of forest duff plus traces of leafmold and wood compost but contained no soil. The amount of sunlight admitted was more than average and was considered adequate for seedling growth. Survival in this group was 84.8 percent and mean growth was 9.80 inches.

Another group had the same environment as that described above except that the plots were built over soil. The mean growth of 9.20 inches was comparable, but seedling survival was poor, being only 27.1 percent. The high mortality in this group cannot be attributed to any definite cause. Seedling mortality occurred throughout the growth period and was not greater at any one time than another. The topography of the soil around the plot permitted good surface drainage. However, it is believed that internal drainage in the plot was inadequate. This seemed to be due to the intimate contact that was established between the soil and layer of duff above it by the activities of earthworms and other soil fauna. Some of the soil was thus brought up and mixed with the duff, and decomposition of the duff was also accelerated.

A third group consisted of three plots built upon a combination of rock and soil and located under greater natural shade than the foregoing groups; otherwise the environment was about the same. Survival in this group was good, being 72.5 percent, but the mean growth of 3.79 inches was poor, being less than one-half that shown in the above groups.

In general the four remaining groups were inferior both in survival, 29.9 to 45.2 percent, and in mean growth, 1.93 to 3.52 inches. Growth was inferior in plots consisting of more than 50 percent of wood compost derived from decayed logs and stumps and also in plots consisting of 10 to 20 percent of soil. The plots constructed over rocks or upon rocks with a thin layer of soil gave a better average survival than those constructed over soil. Seedlings grown with poor drainage but otherwise subjected to conditions for optimum growth were least able to survive.

Sunlight was the most important growth factor. Under the conditions of the experiment, only the first two groups were considered to have received throughout the period adequate sunlight for optimum growth. There was no significant difference between these two groups in growth, but their growth was significantly superior to that of all other groups.

The results indicate the importance of good soil drainage for survival and the importance of adequate sunlight to promote optimum growth after the seedlings have reached a certain stage in their development. It should be mentioned that all of the plots were located in the undisturbed forest, that is, no clearings were made for them. Under these conditions they were subjected to the relatively constant humidity and temperature of the forest atmosphere, and no harmful effect from direct sunlight could be detected. Nevertheless, the estimated amount of direct sunlight as received in some of the plots has been observed to be harmful to seedlings of similar age in the open nursery area. This indicates that the harmful effect of sunlight is due at least in part to its modification of the local humidity and temperature.

In cooperation with William C. Cooper, on assignment to the station from the Office of Foreign Agricultural Relations, experiments were conducted on the effect of nutrient solutions on young *Cinchona* seedlings. A Rubideux Laboratory (*4*)[2] modification of Hoagland's complete nutrient solution, containing a total of 319 p. p. m. of nitrogen supplied by a combination of $(NH_4)_2SO_4$, $Ca(NO_3)_2$, and KNO_3, failed to produce any noticeable effect on the growth of the seedlings. Indolebutyric acid at 10 milligrams per liter was ineffective either alone or as a supplement to the inorganic nutrient solution. Likewise, vitamins B_1, B_2, B_6, C, nicotinic acid, calcium pantothenate, and traumatic acid were ineffective when applied at the rate of 1 milligram per liter. One treatment, which included all the vitamins and 10 amino acids added as a supplement to the inorganic nutrient solution, failed to produce any stimulating effect on the growth of the seedlings.

An organic extract was prepared from dried cow manure that had aged for 30 days, by soaking 1 liter of the material in 1½ liters of water for 15 hours and then straining the water extract through a muslin cloth. When this extract was applied to *Cinchona* seedlings during periods favorable for growth considerable stimulation resulted, and leaves much greener in color than those of the controls were produced. It is well known that the chemical composition of manure is quite complex, and the observed stimulating effect may have been caused by some unknown organic compound. Yet, the greening of the leaves caused by the manure-extract treatment indicated that it was a nitrogen effect, especially since most manures do contain ammonia. Nitrogen in concentrations of 10, 100, 1,000, and 5,000 p. p. m. in the form of ammonium sulfate, sodium nitrate, and ammonium nitrate was later tested on *Cinchona* seedlings growing in the seedbeds at Maricao, but all forms have failed to produce the growth stimulation and greening effect that was induced by the manure extract. The 5,000 p. p. m. solution of all three salts caused severe injury to the plants but no greening. Thus it is certain that the maximum concentration was tested from which a response might be expected.

It appears from these results that the growth stimulation caused by manure extract was not due to nitrogen, at least in the inorganic form, but to some unknown substance.

Coincident with these experiments, a plot of seedlings was treated with finely ground dried cow manure. The seedlings receiving this treatment showed the same growth stimulation as observed for the manure extract. This method gives promise of a more practicable way of applying the manure under large-scale seedbed conditions.

The family Rubiaceae, to which *Cinchona* belongs, is well represented in Puerto Rico, there being some 24 genera and 60 species of trees and shrubs, according to Britton and Wilson (*12, pp. 222–259*). During the summer of 1941 several trial grafts were made with *Cinchona* scions on trees of *Laugeria resinosa* Vahl, *Stenostomum obtusifolium* (Urban) Britton and Wilson, and *Guettarda ovalifolia* Urban, but all of these were unsuccessful.

In August and September of 1942, 2 other species, probably more closely related to *Cinchona*, were selected for trial grafting. Four-

[2] Italic numbers in parentheses refer to Literature Cited, p. 37.

teen scions were inserted on trees of *Exostema sanctae-luciae* (R. & S.) J. Britt, and 20 on *Rondeletia portoricensis* Krug and Urban. Several scions on both species grew for 3 to 4 months, but all eventually died. Essentially the same results were obtained with later trials, but there were indications that improvements in technique might make it possible to graft *Cinchona* successfully on one or both of these species.

During the summer of 1942, 28 representative flowering trees of *Cinchona pubescens* Vahl, *C. officinalis* L., *C. calisaya* Wedd., *C. ledgeriana* Moens, and hybrids between some of these were given a test for self-fertility. This test consisted in enclosing 1 large or 2 small inflorescences on the same tree in bags in such a manner as to prevent the pollination of the flowers from outside sources. As no fruits developed on any of the inflorescenses thus treated, it was concluded that self-incompatibility is normal in *Cinchona*. While this condition makes it impossible to inbreed *Cinchona* by methods that are in universal use on other crop plants, it is of considerable value in cross-pollination when it is desired to produce seeds of known parentage.

Since *Cinchona* is normally self-incompatible, it is unnecessary to emasculate the flowers on the pistillate parent when it is desired to cross two individual trees. The following method of crossing was developed: A tree bearing macrostylous flowers is used as the pistillate parent, if possible, because on these flowers the stigma is either exserted or near the end of the corolla tube, whereas on microstylous flowers it is necessary to remove the corolla and androecium before pollination. Microstylous flowers are easier to manipulate as staminate flowers in the direct application of pollen, because the anthers are near the end of the corolla tube. Inflorescenses are selected approaching the mode of flower maturity, all matured flowers are removed with a pair of small scissors, and the inflorescenses are then bagged as in selfing to prevent contamination from outside sources. Inflorescenses on the staminate tree are likewise bagged. Several daily pollinations can then be made until the pistillate inflorescenses are past their mode of maturity. At the end of the pollination period all unopened flower buds are removed and the bags left on for a few days longer.

In a study of cross-compatibility one inflorescence was selected on each of 22 trees and the flowers were artificially cross-pollinated, using pollen from trees of the same flower type. No fruits developed on any of the inflorescences thus pollinated, although during the same period pollination between unlike flower types resulted in an approximate 75-percent set of fruits.

In these experiments approximately 2,500 flowers on more than 100 inflorescences were pollinated with compatible pollen. The percentage set of flowers pollinated on individual inflorescences ranged from less than 10 to 100. The average for freshly collected pollen was 74 percent. It is evident that early-morning and late-afternoon pollinations gave the best results, while cloudy weather extended this optimum period. Inflorescences located in the shade for most of the day gave a better percentage set than those exposed to the sun. Stormy weather at certain times blew the bags out of position and some of them caught water, which caused the freshly pollinated flowers to mildew. Many of the inflorescences, on which comparatively few of

559850—44——2

the total number of flowers were pollinated, blasted before maturity of the seeds. Certain trees were somewhat more efficient as pistillate parents than others, probably due in part to the size of the stigmatic surface and other morphological characteristics of the individual flowers.

Cinchona pollen is very small and under ordinary methods of handling is killed in a short time by desiccation. When open flowers were collected in the early morning and stored in paper bags in the shade very little of the pollen was viable when used in artificial pollination the following morning. However, when the pollen was stored in stoppered glass vials under the same conditions, approximately 50 percent of the flowers on which it was used the following morning developed fruits.

Since it may often be desirable to cross two trees which flower at different periods, and since the two trees may be at widely different locations, some methods of prolonging the viability of pollen seemed necessary. A preliminary experiment in storage under refrigeration was begun, but it soon became apparent that pollen so stored would not remain viable for more than 3 days. As it is known that the anthers dehisce 12 to 14 hours before the opening of the flower, an experiment was made to compare the longevity of pollen collected in the open flower with that collected in the bud just previous to opening. It was found that pollen in open flowers collected in the early morning and stored in stoppered vials at a temperature of approximately 40° F. gave a 51-percent set of fruit when used after 2 days' storage, and 30-percent set after 3 days' storage. On the other hand, pollen collected in flower buds during the late afternoon previous to the day on which flowers would open, gave a 90- to 95-percent set of fruits when used after 4, 5, and 6 days in the same kind of storage. At the end of 6 days' storage the anthers and corolla had started to disintegrate, which made direct transfer of the pollen somewhat difficult. It was apparent, however, that the pollen remained in good condition even after this period.

Cinchona chemistry: Howard T. Love.

Samples of bark were collected from 29 trees of *Cinchona ledgeriana* and its hybrid varieties growing in the Maricao Forest. All the trees were 10 years old, but varied greatly in size and vigor. The bark was taken from the same relative position on each tree, i. e., at a distance from the ground equal to one-third the height of the tree. The average total-alkaloid content of the bark was found to be 5.76 percent. Nine trees, or 31 percent of the group, contained 6 percent or more of total alkaloids, 7 of the 9, or 24.1 percent of the whole group, contained 7 percent or more of total alkaloids, and 3 of the 7, or 10.3 percent of the whole group, contained 8 percent or more of total alkaloids. The results indicate the importance of including alkaloid content along with vigor and yield of bark as one of the factors in selecting propagating stock.

The bark samples referred to above were classified into 3 groups, thick, medium, and thin. Thirteen thick samples had an average total-alkaloid content of 5.88 percent, 10 medium samples averaged 5.50 percent, and 6 thin samples averaged 5.94 percent. In all 3 classifications there were individual samples with alkaloid contents as low as

4 percent, and 1 sample in each classification had an alkaloid content of 8 per cent or higher, indicating no correlation between thickness of bark and alkaloid content.

In determining total alkaloids in cinchona bark by the volumetric method, the total alkaloids are extracted with a solvent, in this case benzene, and the solvent evaporated to a small volume. An excess of standard acid is added, the remaining solvent evaporated, and the excess acid titrated with standard sodium hydroxide. The amount of total alkaloids can then be calculated from the amount of standard acid used to neutralize them. This procedure is more rapid than isolating the alkaloids in a form pure enough to weigh gravimetrically. The volumetric method is considered to be less accurate, and erratic results were obtained with this method when indicators such as methyl red, bromcresol blue, and mixed indicators were used to determine the end point in neutralizing the excess standard acid.

Good but slightly higher results were obtained with the volumetric method when a pH meter with glass electrode was used to determine the end point in the titration. It was determined that an end point at pH 6.4 at a temperature of 27° C. gave the closest agreement with the results obtained by the gravimetric method.

Lactic acid is a good solvent for the cinchona alkaloids and has the advantage of not being volatilized from boiling aqueous solutions. A 0.2–N solution was used as a standard acid for the volumetric determination of total alkaloids in cinchona bark. Results obtained with the freshly prepared and standardized solution were comparable to those obtained with standard hydrochloric acid. After standing a few days the lactic acid solution became infected with a bacterial growth which increased the acidity of the solution and gave erratic results. It was concluded that the susceptibility of lactic acid to bacterial infection and change outweigh the advantage of its nonvolatility, and that for these reasons it cannot be considered as good a standard for the volumetric method as hydrochloric acid.

INSECTICIDAL PLANTS

Agronomic studies: Rufus H. Moore.[3]

The growing of *Derris elliptica* was extended in the Western Hemisphere from planting material provided by this station during the past year. In cooperation with the Board of Economic Warfare and the Office of Foreign Agricultural Relations 634,310 cuttings were distributed to Argentina. Brazil. Colombia, Costa Rica, Ecuador, El Salvador, Guatemala, Haiti, Hawaii, Honduras, Mexico, and Peru.

In 1940, 9 clones of the Changi No. 3 variety of Sumatran *Derris* were obtained from the Goodyear rubber plantation in Panama. These plants were trellised to promote the development of cutting material, and shield-budding was practiced to facilitate the increase of propagating material of the few original plants in each clone. As a result, sufficient planting material is now available to undertake experimental work. When the clones were from 18 to 21 months old, half of the root systems of 3 plants in each clone was removed. The

[3] Chemical analyses reported in this section were made by Merriam A. Jones, associate chemist.

roots of all clones averaged together contained 7.7 percent of rotenone and 18.7 percent of total extractives. The highest clone analyzed 8.6 percent of rotenone and 20.8 percent of total extractives. These clones were grown on a heavy soil from which similar material of the Sarawak Creeping variety yielded only 4.4 percent of rotenone but when planted on favorable soil produced 7.2 percent. On this basis, the growing of these Sumatran clones on more favorable soil might materially increase the rotenone content of the roots.

Rooted cuttings of a clone of the Changi No. 3 variety of *Derris elliptica* were grown for 13 months in coarse quartz sand in 5-gallon coffee-urn liners in an experiment to test the effect of deficiencies of nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, and iron. Four plants in each treatment were so randomized that each of them constituted a replication.

Hoagland and Arnon nutrient solutions (*8*) were used with ammonium nitrate added to the complete or control solution and to the solution without iron (−Fe), while sodium hydroxide was added to the −N solution, to help stabilize the pH in the sand cultures. Increasing loss of water caused by the progressive development of tops necessitated that the solutions be diluted to one-half strength after 4 months, and to one-third strength after 9 months.

Recognizable and consistent foliar symptoms of deficiencies appeared on the plants in the −P, −S, −Mg, and −Fe treatments. Leaflets of the −P plants were pale green with a bluish-green cast extending a short distance from and along the full length of the principal veins, and the leaves were somewhat reduced in size. Leaflets of the −S plants were a clear lemon yellow. Mesophyll close to the sides of the midrib and between the main lateral veins of the −Mg leaflets became definitely chlorotic, and the leaves were much reduced in size. The lack of iron eventually induced the typical green network of the entire venous system of leaflets, set off distinctly by pale islets of mesophyll tissue. Characteristic patterns of chlorosis were prevented by applying the missing nutrient to newly unfolded leaflets. The lack of nitrogen in the nutrient solution caused no noticeable change in the size or color of leaves. The −K leaves were greatly reduced in size but developed no visible chlorotic or necrotic symptoms. None of the changes in leaflets of −Ca plants were constant enough to be considered definite symptoms.

The total length of stems produced by deficient plants was always less than that of the controls. Plants in the −P, −K, and −Ca treatments were especially stunted, having developed only 5, 2, and 8 percent as much stem length, respectively, as the controls. Treatments repressing stem growth least were −N and −Fe, the plants of which produced 41 and 89 percent as much stem length, respectively, as the controls. Some stems of all plants died. Dead stems of the −Mg and −Ca plants amounted to 54 and 85 percent, respectively; but only 5 to 19 percent of the stems of plants in other treatments died.

The roots of plants in three of the treatments showed physical differences that could be ascribed to mineral deficiencies. The smallest roots of the −P plants were definitely coarse, while those of the −K plants were exceptionally fine. A fairly high percentage of thick roots of the −Mg plants were dead and had to be discarded.

Owing to the fact that in all but three treatments only a few grams of dry roots were secured from each plant, it was not feasible to determine total extractives and rotenone gravimetrically in root samples from individual plants, but such analyses were made of composite samples of all roots in each treatment. For some unknown reason the roots of control plants in this greenhouse experiment averaged only 1.3 percent of rotenone in contrast to the 5 percent that was found in roots of field-grown plants of the same clone. As the gravimetric data were too few in number to be analyzed statistically, the red-color value of each of the 32 root samples was determined with the spectrophotometer. The gravimetric data had the same trends as the colorimetric data.

Percentages of rotenone plus rotenoids [4] expressed on a dry-matter basis would lead to erroneous conclusions, inasmuch as dry matter in the several treatments varied from 8.7 to 20.5 percent. The effects of variation in dry matter, therefore, were eliminated by expressing the red-color value as milligrams per cubic centimeter of fresh root volume. On this basis the concentration of rotenone and rotenoids in the roots of the –S plants was greater by high significance than in roots under any other treatment. The −S roots, with 9.2 mg./cm.^{3}, had 49 percent more rotenone plus rotenoids than the −N roots, 78 percent more than control roots, and 248 percent more than −Mg roots. The withholding of magnesium definitely lowered the red-color value. In addition to the −S roots, the −N, −P, and −Ca roots were superior by high significance to the −Mg roots in red-color value; but the control roots were only significantly superior. The −S and −Mg treatments were the only ones that showed any significance in relation to the control.

The yields of roots are indicative of the type of agronomic fertilizer test that should be undertaken. Plants in the −N, −P, and −K treatments yielded, respectively, 90, 15, and 7 percent as much water-free root tissue as the controls. Withholding nitrogen from the solution applied to the −N plants was largely compensated for by an abundance of large, nitrogen-fixing nodules on the roots. None of the plants in other treatments developed nodules because the nutrient solutions used on them had an abundance of readily available nitrogen. The red-color value of the roots was not significantly affected by the lack of N, P, or K.

To secure information on the adaptability of *Derris* to widely different environments, plantings with from 225 to 275 rooted cuttings of the Sarawak Creeping variety were made in friable soils in each of 7 localities in the central and western sections of Puerto Rico. The slopes of the areas on which most plantings were made varied from less than 1 to 3½ percent, but the land at Cidra had a 9- to 21.5-percent slope at right-angles to the direction of the rows. The plants were spaced 2 to 3 feet, allowed to trail over the ground, and given an application of a 12–8–5 commercial fertilizer at the rate of 800 pounds per acre.

Roots from a border at least 6 feet wide were not included with those of the 100 plants from which yield data were calculated. The

[4] The term "rotenoids" refers to compounds related to rotenone which occur in leguminous fish-poison plants. The red-color value as measured by the Gross-Smith test (*5*) includes rotenone and rotenoids.

depth to which roots were harvested, and hence the yield and quality of the roots, was influenced by local conditions. As an ox-drawn plow was not available at Maricao, roots in the upper 12 inches of soil were dug with shovels. *Derris* in this planting, however, was definitely shallow-rooted like the trees that had been removed to clear the area. At Sabana Grande a layer of rock prevented plowing deeper than 12 inches. Inadequate facilities at Vega Baja and Lajas did not permit harvesting deeper than 10 to 13 inches, respectively. Roots were harvested to a depth of 15 to 16 inches at Cidra, Utuado, and Mayaguez.

Combinations of environmental and harvesting factors operative in each locality varied in such a way that it was difficult to draw precise conclusions from this test.

However, on comparing the data from plantings at different elevations under approximately 80 inches or more of annual rainfall, it appears that elevation was unfavorable to the formation of rotenone. The roots grown at 2,400 feet near Maricao had 2 percent of rotenone, those grown at 1,400 feet near Cidra had 4.3 percent, while those grown at 50 feet near Mayaguez had 6.8 percent of rotenone. Data on analysis of roots according to diameter, not included in this report, show that these differences in quality persisted in roots of comparable diameter and were not caused predominantly by larger proportions of thick roots in the Maricao and Cidra samples.

A comparison of the data for Mayaguez, Utuado, Lajas, and Vega Baja eliminated the factor of elevation and showed a direct correlation between rainfall and root quantity and quality. On practically the same soil, Toa silty, sandy, or clay loam, the yield of roots at Mayaguez under 73.5 inches of rainfall was 1,069 pounds per acre analyzing 6.8 percent of rotenone; that at Utuado, 67.6 inches of rainfall, was 591 pounds containing 6.0 percent of rotenone; and that at Lajas, 66.3 inches of rainfall, 584 pounds with 4.8 percent of rotenone. Although the mean annual rainfall for Vega Baja was only 65.6 inches, there were only 3 months in 28 which had less than 2.6 inches of precipitation. This uniformity in distribution of rainfall and the fine sandy loam soil were principal factors contributing to the comparatively high yield of 977 pounds of roots per acre which analyzed 6.0 percent of rotenone.

A flat area of Toa clay loam at the experiment station at Mayaguez was used for an experiment to test the effect of harvesting *Derris* at 18, 21, 24, and 27 months after planting. Each of the 28 plots was planted to rooted cuttings of the Sarawak Creeping variety to contain 128 experimental plants protected by border plants. A 12–8–5 commercial fertilizer was applied at the rate of 800 pounds per acre 6 months later. The yields of air-dry roots at 18, 21, 24, and 27 months were 828, 934, 1,030, and 1,091 pounds per acre, respectively. The increase for the respective intervals was 106, 96, and 61 pounds per acre. Total extractives and rotenone rose steadily from 17.2 and 5.0 percent at 18 months to 19.2 and 5.7 percent at 24 months, and then declined to 16.2 and 5.2 percent, respectively, at 27 months. Since there was little increase in yield and an actual decrease in quality of roots after 24 months, a crop cycle of this length was indicated for *Derris* under the conditions of this experiment.

Propagation studies: WILLIAM C. COOPER.

William C. Cooper, of the Office of Foreign Agricultural Relations, was assigned to the station to study the effect of growth substances on the propagation of insecticidal plants, particularly *Derris elliptica* and *Lonchocarpus nicou* (Aubl.) DC.

In these studies the alcohol solution-dip method of treatment was commonly used as a method of applying growth substances. This consists of placing the basal end of the cutting to a depth of about 1 inch for a few seconds in a 50-percent alcohol solution containing 1.0 to 5.0 milligrams of the growth-promoting substance per milliliter. Such solutions are considerably easier to prepare than the talc mixtures, the activity of which is dependent upon the fineness of the powder. The solution-dip method of treatment makes application as simple as the powder-dip method, and considerably simpler than the old standard method of soaking the cuttings for 24 hours in dilute solutions of the growth substances. The cuttings used were approximately 10 centimeters long, varied in diameter from 3½ to 4 millimeters, and consisted of a portion of an internode with a node at the apex. The propagation medium consisted of Aguadilla sand (pH 7.0) placed in sash-covered beds in the greenhouse and protected from direct sunlight.

A number of growth substances in solution made up as above in varying concentrations were applied on different dates to over 5,000 small-stem leafless cuttings of *Derris elliptica*. The most effective in inducing roots were 3-indolebutyric acid and 1-naphthaleneacetamide. The minimum effective concentration was found to be about 1.0 milligram per milliliter for both compounds. This concentration produced about 9 roots per cutting as compared to 4 on the controls. However, 2.0 mg./ml. of these compounds produced many more roots, in some instances as many as 100 per cutting. In no instance was injury noted from the use of concentrations up to 5.0 mg./ml. of either 3-indolebutyric acid or 1-naphthaleneacetamide, but very often this concentration caused most of the roots to appear on the stem above the basal cut surface.

1–Naphthaleneacetic acid was found to be effective in inducing root development on *Derris elliptica*, but was effective over a much narrower range than the other two substances. No increased root formation over the controls was noted at 1.0 mg./ml., while at 5.0 mg./ml. a large increase was observed but some injury appeared. It was found that 3-indoleacetic acid and 2-naphthoxyacetic acid were completely inactive at the concentrations tested. The latter compound caused injury at the higher concentrations but did not induce root formation. The potassium salt and ethyl ester of 1-naphthaleneacetic acid showed some root-forming activity at 2.0 and 5.0 mg./ml., but it was less than that observed for the free acid. Both 2,4-dichlorophenoxyacetic acid and 2,4-dichlorophenoxybutyric acid were effective in inducing roots but caused considerable injury at all concentrations above 0.1 mg./ml. The roots formed by these two compounds were abnormally flat, coarse, and short.

The best treatments developed in these greenhouse tests, namely, 3-indolebutyric acid, 1-naphthaleneacetamide, and 1-naphthaleneacetic acid at 2.0 mg./ml., were applied to about 5,000 leafless cuttings just prior to planting in the field.

Data taken on these cuttings at the end of 4 months showed that an average of only 30 percent of the treated cuttings had developed into well-rooted plants with vigorous top growth and that the untreated plants had done just as well. The percentage of cuttings that had died in the treated lots was 40, while in the untreated lots it was only 32. It was apparent, from the results obtained, that whereas the chemicals produced heavy root formation, the plant itself was unable to utilize these roots until such time as adequate top growth had developed. Therefore, the use of root-producing substances on small leafless *Derris elliptica* cuttings cannot be recommended.

The results described above were for leafless cuttings which are easier to handle in the greenhouse and in the field than leafy cuttings, as with the latter there is the problem of keeping the leaves from dying. An investigation was made, however, to compare the root systems produced on leafy and leafless cuttings. In a preliminary experiment, in which only untreated cuttings were used, it was found that after 1 month, leafy cuttings produced much the more extensive root system. The number of principal roots for both was 6, but the average root length was 9.0 cm. for the leafy cuttings as compared with 3.0 cm. for the leafless ones. Likewise, the roots on leafy cuttings were covered with hundreds of fine laterals, while the roots on leafless cuttings had no laterals. The roots of the leafy cuttings were less brittle and there was less breakage in transplanting. After 6 weeks the leafy cuttings had grown normally and showed no setback as a result of transplanting, whereas only about 50 percent of the leafless cuttings remained alive. After 4 months the root system on the leafy cuttings was 3 times as extensive as that on the leafless cuttings.

In more extensive experiments, a range of concentrations of 3-indolebutyric acid was tested on leafy cuttings, and it was found that solutions at 1.0, 2.0, and 5.0 mg./ml. were definitely effective in increasing the number of roots as compared to the controls. The solution containing 2.0 mg./ml. was used both on leafless and leafy cuttings, and this solution induced only 16 roots on leafless cuttings as compared to 34 on leafy ones. When these rooted leafy cuttings were transplanted to the field directly from the sash-covered propagating frames, they grew well and there were no signs of root degeneration.

In addition to the growth substances used above, rooted plants were also treated with solutions of the vitamins B_1, D, nicotinic acid, and calcium pantothenate, and a complex mixture of amino acids at 0.1 mg./ml. None of these treatments appeared to affect root formation, but all appeared to stimulate bud growth during the first 2 weeks. However, after 1 month in the field, shoot growth appeared to be about the same as in the controls.

The rooting of both treated and untreated leafy *Derris* cuttings offers the possibility of utilizing considerable material which heretofore was discarded. This procedure is now being employed to propagate some of the valuable high-yielding clones of Sumatran *Derris*. It is not expected that this method will take the place of using leafless cuttings, but it will supplement the amount of material which can be effectively used to increase valuable clones.

The excellent growth made by treated leafy cuttings indicates that the poor results with treated leafless cuttings were due to the absence

of leaves. It is a well-known fact that the treatment causes a mobilization of food reserves to the base of the cuttings where they are utilized in root formation (*11*, *14*). In treated cuttings where 50 or more roots are frequently initiated on a single cutting there is bound to be a heavy demand on the stored food materials of the cuttings. With leafy cuttings this demand can be taken care of by continued photosynthesis in the leaves. In leafless cuttings there is no way in which to obtain an additional supply of food except by pushing out new shoots. The treatment itself induces temporary bud inhibition, thus the collapse of treated leafless cuttings may be at least partly due to starvation.

The ill effects of chemical treatments on leafless *Derris* cuttings have not been noted on 1-year-old rooted transplants that were removed from the nursery for treatment. Some of these treatments, such as 2,4-dichlorophenoxybutyric acid at 0.1 mg./ml. in 5-percent alcohol for 5 hours, were so potent that an average of 76 roots per plant were induced over a period of 2 weeks. These treated transplants and untreated controls were planted in a field of silty clay loam after 2 weeks' storage. At the end of 5 months an examination of the root systems showed some great differences; the root systems of the treated plants were considerably more fibrous as compared to the controls, which had several vigorously growing roots with only a limited number of smaller roots.

Various kinds of wrapping materials were tested for their possible use in the shipment of *Derris* cuttings. The use of parafilm, pliofilm, and kraft paper stopped moisture loss completely. A colorless waxed paper used locally for wrapping bread was more effective in decreasing moisture loss than the wax paper now being used. The results with the kraft paper were particularly encouraging in that this paper is tough and adapted to shipment of large bundles of cuttings which receive rough handling in transit.

As with *Derris*, cuttings of *Lonchocarpus* treated with hormones showed the same pronounced bud inhibitation and the treatment appeared to be the underlying cause of the deleterious effects. Many roots were induced in both instances, but as with *Derris* new shoot growth was delayed for so long that many of the roots died or failed to develop. With *Lonchocarpus* there was an additional complicating factor in that the delay in new shoot growth on treated cuttings prolonged the period during which the tops of the cuttings were susceptible to decay by a *Penicillium* mold that is prevalent on the Puerto Rico strains of *Lonchocarpus*.

Results obtained in an experiment with clone "C" of *Lonchocarpus utilis* showed new shoot growth beginning on untreated cuttings after 2 weeks, while cuttings treated with 2 mg. per milliliter of indolebutyric acid in 50-percent alcohol showed very little new shoot growth even after 2 months in a muslin-covered propagating frame. The treated cuttings had from 20 to 100 roots per cutting as compared with only 1 to 10 on the controls. However, 60 percent of the treated cuttings, although well rooted, had died from *Penicillium* decay, while only 10 percent of the controls died from this cause. The remaining 90 percent of the controls had an average new shoot growth 68 mm. long.

559850—44——3

It was, therefore, concluded from these results that the hormone treatment should not be used on leafless cuttings of the *Lonchocarpus* being grown in Puerto Rico. Untreated cuttings of this type inserted in a coarse, well-drained sand will usually develop one or more roots after 6 to 8 weeks, and in light of the above experience, this procedure without a hormone treatment is recommended for this type of cutting.

Chemical studies: MERRIAM A. JONES.

A study of a rapid method of measuring rotenone and rotenoids in small samples of insecticidal-plant material was undertaken. Among the methods given in the literature for the measurement of rotenone is the Gross and Smith red-color test as improved by Goodhue (*5*) and by Goodhue and Haller (*7*). The procedure was to add alcoholic KOH containing a small amount of $NaNO_2$ to a rotenone solution and then acidify to develop a red color, the density of which was proportional to the amount of rotenone in the sample. However, the use of this test was at first limited to solutions of pure rotenone because several other compounds occurring naturally with rotenone also give a red color. However, it has been applied to the measurement of the purity of deguelin-CCl_4 solvate (*6*).

This particular colorimetric reaction was chosen for study because Jones et al. (*9*) have shown that the total red color given by derris extracts is more closely correlated with toxicity to houseflies than any of the several other analytical criteria tested. It was hoped that, from this one test, one could obtain the total red color and by photometric analysis thereof arrive at an estimate of the concentration of each of the different compounds contributing to the color.

The details of developing the color were as follows: To 2 ml. of acetone solution of rotenone were added 2 ml. of reagent made by mixing 1 part by volume of 40-percent aqueous KOH with 7 parts by volume of ethanol containing 1 gram of $NaNO_2$ per liter. After 5 minutes, 5 ml. of H_2SO_4 (1:3) were added, whereupon the red color appeared.

The red color was analyzed in a Coleman double-monochromator spectrophotometer using a 30 mμ exit slit. By measuring the transmittance of the red solution as compared to a blank, at regular time and wavelength intervals, a complete spectrogram from 280 to 1,000 mμ of the red color at 15 minutes after mixing was constructed. For colorimetric analysis of rotenone one should read the transmittance at 540 mμ, because to this light the red solution is most opaque. In other words, at 540 the test is most sensitive, in that a given amount of rotenone will lower the transmittance of the test solution to a greater extent than at any other wavelength.

Temperature proved to be a critical factor. Although sub-room temperatures gave considerably lower transmittances, such temperatures are more difficult to maintain with an ordinary thermostat; so, for convenience, a standard temperature of 30° C. was chosen.

The manner in which the acid was added to the test solution also affected the results. It was found that measuring the acid into a test tube and inverting the tube into the test solution in a 50-ml. conical flask while swirling yielded the most precise results. The precipitate formed upon acidification must be quickly dissolved by rapid addition of the acid. This precipitate was also obtained under the same conditions when alcohol without $NaNO_2$ and acetone without rotenone

were used. The absence of either the sulfate ion or the potassium ion resulted in no precipitate. Therefore, the precipitate formed upon adding the acid was K_2SO_4 or $KHSO_4$ and only dissolved when the water concentration became sufficiently high.

No significant effect on the test was found when the color or intensity of the light falling on the solution was changed. It was found that the KOH–$NaNO_2$ reagent was stable for several weeks if the alcohol used was previously purified over KOH and zinc.

In trials with 2- to 7-ml. portions of the acid to develop the red color, it was found that the resulting transmittance increased markedly with the amount of acid used, excess acid making the test less sensitive because less red color was produced per unit of rotenone. On the other hand, small amounts of acid resulted in difficulty in dissolving the evanescent white precipitate formed upon acidification. The maximum extinction per unit volume was found when 4 ml. of acid were used, but since the precipitate was somewhat persistent it was decided to use 5 ml. to obtain optimum color without excessive persistency of the precipitate.

The effect of the time allowed between mixing the sample with the KOH–$NaNO_2$ reagent and subsequent acidification was also studied. Using a solution of rotenone in acetone and an acetone extract of derris powder, it was found that as the time between mixing with the reagent and acidification was increased from 0.5 to 3 minutes the resulting transmittance decreased. With intervals of from 3 to 6 minutes a minimum transmittance was obtained. As more than 6 minutes were allowed the transmittance began to increase. Therefore the maximum sensitivity of the test was attained when the time interval was from 3 to 6 minutes. It was further found that the differences due to changing the time from 5 to 4 minutes or 6 minutes were of the order of magnitude of the error of the instrument. Therefore the time of acidification after mixing was not a critical factor and may be set as 5 ± 1 minute without significantly affecting the results.

Following the above modifications of the Gross-Smith test, the extinction coefficients of the red color given by rotenone, deguelin, elliptone, tephrosin, and toxicarol at 15 minutes after mixing the acetone extract and the KOH–$NaNO_2$ reagent were measured at numerous wavelengths. Using synthetic mixtures and solving for concentrations of the separate compounds by means of simultaneous equations gave satisfactory results for knowns containing 2 or 3 compounds but failed completely for derris extracts. This was very likely due to the presence of other substances in the derris extract which contributed to the red color and also to changes in the substances upon purification, so that the extinction coefficients for some of the compounds when isolated were not the same as when bound up in the resin.

Some data have recently been obtained correlating the red-color value determined as outlined above with the rotenone content as determined by the Official Method (*1, pp. 64–66*). Comparisons were made in 59 samples of derris root from 3 different sources. The roots were ground in a Wiley mill through a one-half-millimeter sieve and portions analyzed gravimetrically. Small amounts of about 500 mg. were macerated in 50 ml. of acetone with intermittent swirling for 16 hours, chilled, and filtered. An aliquot of the filtrate was diluted to 25 ml. with acetone and analyzed colorimetrically with the thermostat set

at 30° C.±0.1. It was found that this procedure extracted as much of the rotenone and rotenoids as exhaustive extraction in Soxhlet apparatus, but less total acetone extractives. With Soxhlet extractors 6 hours' extraction was not so complete as 12 hours followed by standing overnight before removal. By maceration 15 minutes was sufficient time for 87 percent extraction and 3 hours for 94 percent extraction.

Seven samples from regional test plots gave ratios of rotenone (gravimetric) to rotenone plus rotenoids (colorimetric) ranging from 0.343 to 0.387 and averaged 0.364 ± average deviation of 0.012. The ratios for 29 samples from an age-at-harvest experiment varied from 0.360 to 0.427, averaging 0.390 ± average deviation of 0.013. In 23 samples from Guatemala the ratios varied from 0.082 to 0.529, averaging 0.369 ± average deviation of 0.089. However, it is important to add that here the results for rotenone as determined gravimetrically were somewhat in error because of the low rotenone content of many of the samples; for the sample which had the ratio 0.082, the rotenone content was 0.22 percent. The ratio is, of course, most sensitive to error at small concentrations. Considering only the 14 samples having over 1.5 percent rotenone, dry basis, the average ratio was 0.438 ± average deviation of 0.055.

From these results it was concluded that the red-color test could be used for plant selection with entirely satisfactory results. The problem in plant selection of rotenone-bearing plants resolves itself into finding a rapid analytical method which can be applied to large numbers of samples. The necessity of accepting some error is compensated for by the possibility of testing large numbers of separate samples. Moreover, considering the large variation due to the difficulty of selecting a representative sample from the root system and the marked effect of environmental conditions on the rotenone-bearing ability of a clone, it would appear that the colorimetric procedure, more economical of time and labor than the gravimetric method, is sufficiently accurate to be used for analyses in connection with the selection of high-rotenone clones. A factor of 0.4 could be used or the selection made on the basis of the red-color value. Thus many low-rotenone clones could be discarded without resort to the comparatively expensive gravimetric assay. Promising clones thus selected could then be propagated under known conditions and later checked for rotenone content by the gravimetric procedure.

Freshly harvested insecticidal plant roots such as those of *Derris* and *Lonchocarpus* contain about 66 percent water. A simple method for drying the roots on a large scale for storage and shipment is essential. During drying three separate processes of interest may occur in the fresh root: Evaporation of water, degradation of rotenone and related compounds by the air and light, and the consumption of carbohydrate reserves by respiration. A simple drying experiment to measure the practical effect of these changes was undertaken. Duplicate samples of the roots of *Derris elliptica* var. Sarawak Creeping, from 3 to 10 mm. in diameter and chopped into 2-cm. pieces, were used to test three methods of drying—sun, shade, and oven. A moisture determination on the original lot was also made. The oven-dried samples were held in an electric oven at 80° C. for one-half hour and then at 55° for 16 hours. After the sun- and shade-dried samples had reached constant weight they were finished in the oven in the same

manner. In the sun the rate of drying reached a maximum on the second day and then decreased, while in the shade the maximum was obtained on the third day. A constant weight was reached in the sun on the fifth day and in the shade on the seventh day. All the samples were ground in a Wiley mill through a ½-mm. sieve and stored in a desiccator for 1 week before being analyzed in duplicate for moisture and rotenone (*1, pp. 64–66*). The results as averages of duplicate analyses of duplicate samples for rotenone content were: Sun 7.99 percent, shade, 7.97, and oven 7.65 percent.

It would be expected that in shade drying the rotenone percentage would be higher than in sun drying because of greater carbohydrate utilization and less rotenone degradation. However, since the differences were within the experimental error, it was concluded that the method of drying had no effect on the rotenone content. For practical purposes it appears that any of the three methods of drying would be satisfactory, depending upon facilities available and existing field conditions.

Several parts of the yam bean plant (*Pachyrhizus erosus* Urban) gave a positive reaction with the Durham test. The best test was given by the mature seed. Application of the test to the split seed showed that the compounds responsible for the reaction were present in the hypocotyl and the vascular tissue. The principal parts of the plant were analyzed by the red-color test. If the red color was due to rotenone and related compounds, these were found to the following extent on a percentage dry basis: Stem 0.03, leaf 0.11, pod 0.02, seed 0.66, and tuber none.

VEGETABLE CROPS

Seed production: Claud L. Horn and David L. Stoddard.

The planting of vegetable crops to provide seed for the food-production program of the island was continued in conjunction with the Work Projects Administration and War Emergency Program. During the course of the year 2,132 pounds of seed of the USDA–34 variety of sweet corn were distributed, of which some went to the military forces. Seed of this sweet corn was also distributed to the Canal Zone and the Virgin Islands and to the following foreign countries: Argentina, Costa Rica, Cuba, Ecuador, El Salvador, Guatemala, Honduras, Peru, Tahiti, and Trinidad, B. W. I.

A quantity of yams, *Dioscorea* sp., totaling 16,531 pounds, particularly of the Morado and Potato varieties, were made available to the W. P. A.

The Seminole variety of soybean continued to show promise, and seed totaling 1,072 pounds was distributed through the W. P. A. and the Extension Service of the University of Puerto Rico. From the standpoint of production and food value, soybeans offer a high return per acre in comparison with other leguminous crops that can be grown in Puerto Rico.

Soybeans for cooking tests, both in the green and dried state, have been made available to various individuals, and in addition considerable amounts have been supplied to the W. P. A. and the Extension Service for use in school lunchroom projects and home economics departments. A test of acceptability by the local people was carried

out in conjunction with the W. P. A. School Lunchroom Project in two schools in the Mayaguez area, one urban and the other rural. In the urban school 62 percent of the children consumed all of the serving of soybeans given them, and 80 percent consumed over 60 percent of the amount served. In the rural school the percentage was somewhat less, and in no case did any one of the children consume all of the serving offered; however, half of the children consumed 75 percent of the beans served.

Field studies: Wallace K. Bailey.

In a size-of-seed-tuber test with the Potato 44 variety of yam, in which 6 sizes of tubers, ranging from an average of 4.4 to 126.6 grams each, were replicated 6 times in a randomized block arrangement, the yield of tubers was found to be correlated with the size of the seed planted. Each replication consisted of 3 adjacent rows 2½ feet apart and 26 feet long with hills 2 feet apart in the row. Two hills at each end of each row were discarded at harvest to reduce border effect, leaving 27 hills per seed-tuber replication on which records were taken. Seed consisted of whole tubers that had been stored in an open shed for 4 months. The tubers were planted in ridged rows and the vines trained to wooden stakes 6 to 7 feet high. The planting was made on the heavy clay loam soil of the station lowlands.

The yield of tubers varied from an average of 643 grams per hill from the smallest seed to 2,626 grams per hill from the largest, equivalent to 12,350 and 50,437 pounds per acre, respectively. The increases obtained with successively larger seed tubers were significant, but the yield was not in direct proportion to the size of tuber planted. The average yield per pound of tubers planted was greatest for the smallest seed and decreased progressively from 146.1 pounds to only 20.7 for the largest.

The average increased yield per additional pound of tubers planted over the yield from the next smallest seed tubers is the most critical test of the efficiency of the seed. Efficiency in this regard decreased from 85.6 pounds for seed averaging 8.9 grams each to 5.8 pounds for seed averaging 126.6 grams. It seems probable that under the conditions of this experiment profitable returns would not have been obtained with seed much larger than 126.6 grams.

There are two practical conclusions which can be drawn from these tests. Where yield per acre is the primary consideration the use of seed as large as one-fourth of a pound can be expected to give profitable returns. Where increasing the available planting material is the primary consideration, much smaller seed can be used to advantage; tubers so small that they would normally be left in the field at harvest would be satisfactory for such a purpose.

PLANT INTRODUCTIONS

Field studies: Claud L. Horn and Milton Cobin.

While transportation difficulties curtailed importations, a sizeable list of plants, amounting to 168 species and varieties, were introduced during the fiscal year, mostly through the cooperation of the Division of Plant Exploration and Introduction of the Bureau of Plant In-

dustry, Soils, and Agricultural Engineering. Of these, 100 were classified as economic and included food, drug, insecticidal, fiber, and rubber plants. Among the more interesting introductions were seeds of the Carob bean, or St. John's bread (*Ceratonia siliqua* Linn.), received from Bermuda. It is hoped to establish plantings of these trees, which produce an abundance of edible pods, in the arid pasture areas on the south coast of the island. Seeds of the Calabar bean (*Physostigma venenosum* Balfour), indigenous to West Africa, were obtained and readily germinated following scarification. The commercial supply of the beans, from which an important drug is obtained, is said to be nearly exhausted. A number of new grape varieties, hybridized in southern Florida by Joseph L. Fennell of the Office of the Coordinator of Inter-American Affairs, were turned over to the station on his departure. Several of these grape varieties have already been tested and proven of value in southern Florida and now are being tested as to their adaptability to Puerto Rican conditions. A total of 350 vines were planted on sloping terraced land of Nipe clay soil on the station property at Las Mesas at an elevation of about 1,000 feet.

Increased facilities for plant propagation and additional labor provided by the War Emergency Program of the Insular Government have made possible expansion of the plant propagation project. The demand for plant material by the Army and Navy continued to be great, and considerable material was also requested by other Federal and Insular agencies. Of 189,133 plants distributed on the island, 43,678 were supplied to the military forces. In addition, large quantities of seed and grass sod and runners were also distributed. A considerable number of requests were received for seed and planting material of various economic and ornamental plants from foreign countries, including Argentina, Brazil, Colombia, Costa Rica, Cuba, Ecuador, El Salvador, Guatemala, Haiti, Honduras, Jamaica, Mexico, Peru, St. Lucia, Tahiti, and Trinidad.

ENTOMOLOGY AND ECONOMIC ZOOLOGY

General investigations: Harold K. Plank.

Under war conditions, the prevention of the destruction of food crop seeds by insects is imperative. Early fumigation of corn and subsequent isolation of the grain is effective, but such a method is both expensive and hazardous. A study was therefore made of the effectiveness of methods that might be cheaper and safer, as well as more usable by the small farmer. Copper carbonate dust, as used on dried, shelled grain for smut control, has been reported to give protection against insects that attack cereal seeds. More recently, some inert dusts have been similarly applied to control the rice weevil and other insects in stored grain.

An experiment was set up to compare 4 dust treatments with carbon-disulfide fumigation and no treatment. Insect attack was determined at biweekly and monthly intervals by examining 100 grains of corn from each of the 4 replicates of each treatment. Most of the damage found was caused by the rice weevil (*Sitophilus oryza* (L.)). The square-necked grain beetle (*Cathartus quadricollis* (Guér.)[5] was

[5] Determined by M. S. Fisher, Bureau of Entomology and Plant Quarantine.

next in abundance. A few specimens of the bamboo powder-post beetle (*Dinoderus minutus* (F.)) were found, and evidence of its feeding was quite general but did not exceed 4 percent in any one sample. Larvae of the rice moth (*Corcyra cephalonica* (Stainton)) became abundant after several months' exposure, and the Indian-meal moth (*Plodia interpunctella* (Hbn.)) appeared later in the grain that had been badly damaged by other insects.

Two treatments were applied by rolling the newly harvested seed ears in copper carbonate dust, diluted with hydrated lime to 83 percent of the mixture, and lime alone when the grains had separated sufficiently for the dust to penetrate to the base of each grain. In the case of the two treatments applied to shelled grain the dust was simply mixed thoroughly with the seed. The dusts applied to shelled grain were relatively ineffective, the corn under these treatments being infested to the extent of 72 and 62 percent 3 months after harvest. When the ears were rolled in the dusts the infestation was held to about 15 percent during the first month, and below 50 percent for 3 months. Fumigation was the only treatment effective in preventing serious damage for a longer period than 3 months. Viability decreased at an accelerated rate as infestation increased. However, the dusted grains maintained high viability for a period up to 3 months. It was concluded that fumigation with carbon disulfide gave the best results, but rolling the seed ears in hydrated lime at the time the grains separated prevented early infestation by storage insects while maintaining viability of the seed. This method can be recommended as a simple and readily usable way of preserving corn seed for at least 3 months after harvest.

There are few pests of planted, growing, and stored food crops that are more destructive in their habits or harder to control than rats. The station frequently has experienced considerable interference with the seed-production program from this source. Areas near favorable cover, such as fence rows, ditch banks, or headlands and terrace banks covered with dense grass, have produced spotty stands because rats have dug up the seed and later, in the case of corn, eaten into the ears that developed.

All 3 kinds of rats that are common in the continental United States occur in Puerto Rico, namely, the brown or Norway rat (*Rattus norvegicus*), the black or ship rat (*R. rattus rattus*), and the brownish-gray, Alexandrian, or roof rat (*R. rattus alexandrinus*). To gather data on the distribution and relative density of the population of each kind, snap-type traps were kept baited with fresh, dry coconut near to or in station buildings where rats were known to be plentiful. Of the total of 63 rats caught during 8 consecutive months 1 was the Norway rat, 4 were black rats, and 58, or 92 percent, were brownish-gray or Alexandrian rats. Of the total caught, 38, or 60 percent, were trapped in farm buildings and 25, or 40 percent, in dwellings. The brownish-gray rat was by far the commonest kind found in both locations.

Ground-corn and corn-meal baits poisoned with precipitated barium carbonate were modified by the addition of grated dry coconut to increase their attractiveness to rats infesting station fields being planted with corn. Various formulas were tried, but the greatest take was obtained by using a combination of one-half corn meal, one-sixth

grated coconut, and one-third cracked corn poisoned with barium carbonate at the rate of 6 parts by volume of bait to 1 of poison. After moistening with water, heaping-teaspoonful quantities were wrapped in individual packets of medium-weight waxed paper and distributed at 6-foot intervals along the edges of the fields. Two-thirds of all the packets distributed, 80 percent of those containing coconut and 52 percent of those composed of corn meal alone, were either partly consumed or taken away entirely. The greatest takes in all trials occurred near heavily grassed ditch banks or other cover.

After several days' exposure it was noted that the baits containing coconut became infested by fire ants (*Solenopsis geminata* (F.)). However, it was estimated that any repellent effect that such infestation may have had was overcome largely by placing the baits late in the afternoon. The stand of corn obtained, even near dense grass cover and where fire ants were present, was nearly 100 percent, whereas previous plantings in the same vicinity had always been spotty.

Following the procedure described in previous reports, five more introduced bamboos with promising usefulness for construction purposes were tested for susceptibility to the bamboo powder-post beetle (*Dinoderus minutus*) in comparison with the common bamboo (*Bambusa vulgaris* Schrad.). Standardized test rings were taken from the bottom, middle, and top internodes of one culm from five separate clumps of each species. All culms had completed or were completing their first year's growth. The number of beetle attacks found on the test pieces at the end of 1 month's exposure was used to determine the susceptibility in relation to that of *B. vulgaris*.

The relative susceptibility of *Bambusa textilis* McClure was found to be 0.27 percent, which is the lowest recorded for any species thus far tested. *Sinocalamus oldhami* (Munro) McClure (*Bambusa oldhami* Munro)[6] with 1.52 percent relative susceptibility, was next. *B. longispiculata* Gamble ex Brandis was 6.23 percent and *D. membranaceus* Munro 8.99 percent susceptible both of which can be considered low. The striped variety of the common bamboo, *B. vulgaris vittata* A. & C. Rivière, was highly susceptible, 44.18 percent. As in previous tests, rings from the bottom and middle internodes sustained more beetle attacks than those from the top.

The presence of starch in the wood of the various species was determined visually by the iodine test. In general, the rings showing the strongest and weakest reactions were the ones from the internode positions attacked the most and least, respectively, by the beetle. Outstanding was the fact that few of the pieces of *Bambusa textilis* and *Sinocalamus oldhami* tested with iodine showed the presence of starch, and despite the fact that the wood of both species, particularly of the latter, was comparatively soft, all parts of the culms of these species were attacked less than those of any other. Pending a study of the influence of the physical condition of the wood, it is apparent that starch content was an important factor in determining susceptibility.

Biological control activities: Kenneth A. Bartlett.

A number of shipments of parasites were received from the South American Parasite Laboratories of the Bureau of Entomology and

[6] This introduction, P. I. No. 76496, recently determined as above by F. A. McClure, research associate, United States National Museum, was formerly carried under the name *Dendrocalamus latiflorus* Munro.

Plant Quarantine. A total of 7,889 puparia of *Paratheresia diatraeae* (Brèthes), a fly parasite of the sugarcane borer (*Diatraea saccharalis* (F.)), was sent from Brazil. Unfortunately one large shipment was delayed in transit, and practically all of the material arrived dead. The total emergence of all shipments received was 3,398 adults. Liberations of *P. diatraeae* from these shipments and subsequent rearings were made in February and March at Hormigueros and Santa Isabel, 1,191 at the former locality and 2,287 at the latter.

Consignments of two other sugarcane-borer parasites were included with the *Paratheresia* material. From one, *Metagonistylum minense* Towns, 173 adults and from the other, *Ipobracon amabilis* (Brèthes), 11 adults were liberated at Hormigueros.

Nine shipments of two species of parasites, *Acaulona peruviana* Towns. and *Hyalomya chilensis* Macq., which attack cotton stainers, *Dysdercus* spp., were received from Peru. These fly parasites were shipped in the pupal stage, and despite delays in transit generally arrived in good condition, although in some shipments considerable emergence had taken place. From a total of 2,486 good puparia received there was an emergence of 963 *H. chilensis* and 793 *A. peruviana*.

As has been reported previously, oviposition was not obtained in the laboratory with *Hyalomya chilensis*, but *Acaulona peruviana* readily oviposited in the 2 common native species of *Dysdercus*, *D. andreae* (L.) and *D. neglectus* Uhler. A total of 915 nymphs and adults of *D. neglectus* was parasitized in the laboratory and then liberated in the field. Liberations of the adult flies were made at 2 locations, 125 *A. peruviana* and 809 *H. chilensis* at Guayanilla and 495 *A. peruviana* at Isabela.

A number of requests were received for parasites and predators of injurious insects. Adults of 3 species of scale predators recently introduced by the station and which have become well established in Puerto Rico were sent to the Canal Zone. The shipment consisted of 800 *Chilocorus cacti* (L.), 550 *Cladis nitidula* (F.), and 600 *Egius platycephalus* Muls. According to reports received the number that died in transit was negligible. Another shipment consisting of 675 *E. platycephalus* and 450 *C. cacti* was sent to Trinidad, B. W. I., where they arrived in excellent condition.

In addition to the above shipments, 367 larvae and 660 adults of the predaceous elaterid *Pyrophorus luminosus* (Ill.) were sent to Florida in an attempt to effect their establishment there for the control of white grubs. These larvae were collected through the cooperation of the agent of the Extension Service of the University of Puerto Rico at Cayey.

A shipment of the giant toad, *Bufo marinus* L., was also sent to Florida for trial. Previous shipments of these toads have been made to the southern continental United States, but the introductions thus far have not met with success.

CHEMISTRY

Plant products: José O. Carrero.

Due to economic circumstances, the diet of a large majority of the island population has been influenced by the necessity of growing high-priced agricultural crops for export and in return importing

staple foods of comparatively low value, such as rice and beans, without consideration of nutritive values. While the use of rice and beans in the Puerto Rican home may be supplemented to some extent by locally grown vegetables, it can be conservatively stated that a high proportion of the diet of 90 percent of the population consists of rice and beans. Such a diet, of course, provides much starch, little fat, and only a medium to fair amount of protein.

The peoples of the Far East have depended for hundreds of years upon a legume, the soybean, as their chief source of food. In the United States, the planting and use of this legume as food for both man and animals has increased tremendously during the last 10 years and is now maintaining a position of major importance in continental agriculture.

In 1919 this station was awarded a gold medal at the local agricultural fair for an exhibition of soybeans. However, apparently little was done with the crop thereafter, and only recently has it begun to receive the attention of the agricultural workers of the island. During the past several years the station introduced a variety of soybean known as the Seminole which thrives well under Puerto Rican conditions and is considered to be a high producer in comparison with other leguminous crops commonly grown on the island. In order to obtain for comparison actual figures on the nutritive values of various leguminous seeds grown in Puerto Rico or introduced from other sources into the local market, samples of such legumes were obtained as actually sold, and these and soybeans grown at the station were analyzed in duplicate to determine the content of the various constituents on a moisture-free basis. Comparisons were made of soybeans with the native white bean and pigeonpea (or "gandul"), and the imported white (or navy), pink, and red beans, cowpea ("frijol"), and canned garden pea.

While the protein content of all the legumes examined can be considered high, it was found that the soybean protein was almost double that of each of the others. Other workers report that the protein of the soybean is a good although not complete substitute for meat, eggs, and milk, which, of course, are foods having the highest known protein quality for the human diet. The fat content of the soybean was found to be more than 10 times that of the other legumes with the exception of the pigeonpea, in which the value was 6 to 7 times as great. The degree of digestibility of the fat in the soybean is considered high and comparable with other fats usually consumed. The soybeans analyzed were unique in that they had a higher content of total sugars and a lower content of starch and total carbohydrates than the other legumes. Since the soybean is used both as a fresh, green vegetable and as a dried bean or in the form of milled flour, it is important to note that on a moisture-free basis little difference in nutritive value was found between the immature beans and the naturally dried ones.

Avocado oil studies: Howard T. Love.

In connection with a study of the availability of the oil in avocado fruits, sections and smears of the fresh pulp were stained with Sudan III and Delafield's hematoxylin solutions. Microscopic examination of the stained specimens showed that the cell nucleus was attached to one side of the cell wall and occupied a very small portion of the cell cavity. The remainder of the cell cavity was filled with a fine emul-

sion of water and oil. The cell walls, defined by the colored emulsion, were only slightly colored by the hematoxylin, which indicated that they are not entirely cellulosic but also contain pectins. This view was further substantiated by a positive pectin test in the pressed residue.

Examination of similarly stained sections and smears of pulp treated with unslaked lime showed many collapsed cells. The collapse of the cells appeared to have been due at least partly to disintegration of the cell walls by the lime. The consequent release and breaking of the emulsion was evidenced by the appearance of patches of water and of oil on the surface of pulp treated with unslaked lime.

A sample of the flesh of West Indian avocados analyzing 7.6 percent of oil was treated with unslaked lime at the rate of 3 parts of lime to 100 parts of flesh. After standing for 1 hour 5.4 percent of oil was obtained by expression. After repacking and pressing, an additional 1.4 percent of oil was obtained, which brought the total yield to 6.8 percent, or about 90 percent of the total oil present. It is probable that even better yields could be obtained with a good filter press, since the pressed cake still contained 20.6 percent oil and 53.0 percent water.

When the treated pulp was pressed immediately after the lime treatment a light-green oil was obtained, while if the mixture was allowed to stand for 1 hour before pressing a golden-yellow oil resulted. Three successive extractions of the cake with petroleum ether yielded 93.6 percent of the remaining oil, which was dark green. The green oil as well as the yellow oil can be decolorized with charcoal.

West Indian avocados had a much lower oil content than the Guatemalan and Mexican. Most commercial varieties of the two latter groups are said to contain 15 percent or more oil. The oil content of the Puebla, a Mexican variety, is reported to have exceeded 25 percent, and the Fuerte, a Guatemalan × Mexican hybrid, is reported to have reached a maximum of 30 percent of oil. A grower intending to utilize avocados for oil production should give careful consideration to the high-oil-yielding varieties.

Treatment of the pulp of West Indian varieties of avocados with unslaked lime to facilitate the extraction of oil showed that significantly larger portions of unslaked lime were needed than for the Spinks variety used in previous tests (*10*). In the West Indian varieties 2½ to 3 parts by weight of unslaked lime per 100 parts of pulp were necessary to produce a mixture from which the oil and water could be readily pressed, whereas 1 part per 100 gave good results with the Spinks variety. This difference was probably due to the higher water content and the smaller amount of crude fiber or residue found in the West Indian varieties. Fruits of the West Indian varieties contained 6 to 8 percent oil, 85 to 90 percent water, and 3 to 5 percent crude fiber; while the Spinks variety contained 15 to 22 percent oil, 70 to 80 percent water, and 6 to 9 percent crude fiber.

Miscellaneous: Howard T. Love.

In cooperation with the Puerto Rico Development Co., a paint pigment was prepared from the reddish-brown, lateritic soil of Las Mesas known as Nipe clay, which is high in ferric oxide. The pigment was prepared from screened soil by ignition and grinding dry in a ball mill. Tests showed that 6 to 8 hours' grinding was almost as effective as 24 hours'. Regrinding 4 parts of the ground soil with 6 parts

of linseed oil in a ball mill for 6 to 8 hours gave a paint ready for use on thinning with linseed oil.

In another method of preparation the soil was thoroughly washed with water and ground for various periods of time from 6 to 24 hours; grinding 8 hours gave a satisfactory pigment. The soil was then air-dried for a week or more, or oven-dried for 24 hours. The resulting cake of pigment broke up readily when ground with linseed oil in a ball mill. This paint was lighter and not so bright in color as that prepared by ignition.

The prepared pigment analyzed 52 percent ferric oxide. Preliminary tests indicated that this paint would be satisfactory as a general outside paint for roofs, barns, boats, and machinery.

The dried whole coffee bean, after the removal of the fleshy pericarp, consists of an endocarp or parchment shell, an inner seed coat known as silverskin, and finally, innermost, the endosperm, or coffee bean, of commerce. The hard parchment and silverskin are removed by milling, and this mixture is a waste product. An analysis of the parchment showed a caffeine content of 0.30 percent, and of the silverskin, 0.83 percent. Silverskin comes from the mill in a finely divided state suitable for extraction without further grinding. This waste product of coffee industry may be of value as a source of caffeine when additional sources are needed. Barrett (*2*, *p. 77*) states that silverskin contains 10 to 11 percent protein, 35 percent carbohydrates, and 36 percent fiber, and suggests that it could be used in stock feeds.

AGRICULTURAL ENGINEERING

Miscellaneous: Barton C. Reynolds.

The replacement of termite-infested doors and windows throughout the administration building of the station with native hardwoods was carried out with special funds provided for this purpose. Similarly, three of the station houses were completely renovated and put into good condition.

A coffee drier which had not been in use for many years was relocated and connected to a large, specially constructed wooden cabinet for the drying of seeds and other plant material. The cabinet was divided into three compartments each with seven removable shelves. In each compartment a different degree of heat may be admitted, or each can be used separately as desired. The drier, outfitted with steam coils, was remodeled in such a manner that small quantities of seed may be dried quickly within the machine itself without using the cabinet. The drier and cabinet provide very much needed equipment for the drying of seeds and plant material, particularly useful in the present production of large quantities of vegetable seeds for the food-production program.

A considerable number of shop tools, consisting of bench saws, planes, and lathes, were transferred to the station by the United States Army from property formerly utilized by the National Youth Administration in woodworking shops throughout the island. This new woodworking equipment has been installed in the bamboo shop and adds materially to the machine tools now available for both bamboo and general carpentry work.

In cooperation with the W. P. A., the construction of a new air-conditioned greenhouse for work with *Cinchona* was undertaken. This

greenhouse provides three chambers which may be controlled at different temperatures and humidities for experimentation on the growing of *Cinchona* seedlings and other plants. Another greenhouse was completely renovated and glazed, and new plant-propagation benches were installed to provide for work being undertaken on growth-promoting substances with *Derris* and *Lonchocarpus*.

Much necessary supervision was given to the installation, maintenance, and repair of laboratory equipment and other machinery, and to surveying and construction work, all vital to the carrying out of the various projects of the station.

BAMBOO

Propagation and distribution: Claud L. Horn, Armando Arroyo, and Milton Cobin.

Considerable interest in the bamboo project has developed as a result of the impetus given the program by the Puerto Rico Development Co., through the efforts of which the first commercial manufacture of bamboo furniture on the island has been realized. The total distribution of bamboo plants during the year was 3,790, which is larger than the combined distribution for all previous years. Eight species were represented, of which 2,218 plants were of the principal industrial species, *Bambusa tulda* Roxb.

Over 23,000 feet of clump-cured canes of *Bambusa tuldoides* Munro were supplied to the Navy for construction purposes, and smaller amounts were supplied to the Army and Marine Corps.

Requests were filled from Cuba, Jamaica, Trinidad, and Ecuador for planting material of the introduced species.

Increase plantings totaling about 2 acres were established during the year for *Dendrocalamus asper* (Schultes) Backer (*Gigantochloa aspera* Hort.), *G. verticillata* (Willd.) Munro, and *Bambusa textilis*, three species of great utility in their native habitats.

Growth studies: Milton Cobin.

A small planting of the Japanese timber bamboo, *Phyllostachys bambusoides* Sieb. & Zucc., made at the station during 1935, has during subsequent years produced shoots which were stunted and herbaceous in appearance. During the growth flush of 1943 many of the new culms grew to a height of over 15 feet and a diameter of approximately 1 inch. This bamboo is reported to require 12 to 15 years under favorable conditions before anything approaching maximum development can be expected. On the basis of the comparatively vigorous growth produced during this season, it is hoped that this temperate-zone, running-type bamboo may prove adapted to Puerto Rico.

Data on the growth of *Bambusa tulda* were obtained from a planting made during October and November 1937 on a heavy Catalina clay soil located on a hillside of the station grounds. The culm stumps were planted approximately 20 feet apart and have not received any treatment or cultivation since establishment.

Analysis of 40 clumps showed a decrease in the annual culm production during the season of 1942 as compared with the culm production for 1941 which in turn was lower than that of 1940. The average

number of culms produced per clump was 6 in 1940; 5 in 1941; and 4 in 1942.

A considerable number of shoots produced during the 1942 season failed to mature, only 45 percent of those that sprouted developing into full-sized culms. The remainder of the shoots ceased growth upon reaching about 1 foot in height.

The diameter [7] of the culms in 25 of these clumps averaged 1.31 inches in 1940, 1.77 in. 1941, and 1.80 inches in 1942. Although the average diameter for each new culm increased with each year's culm production, the aggregate of the diameters for 1942 was lower than for the preceding 2 years because of the decreased production of culms during that year.

Utilization: Allan Gould and Rafael Fantauzi.

For a part of the year the Office of the Coordinator of Inter-American Affairs cooperated in the program of utilization of bamboo and sponsored the employment of Allan Gould as designer. This cooperative work was later taken over by the Puerto Rico Development Co. Several new and interesting designs have been completed in which types of construction and joints that are particularly adapted to bamboo have been utilized. The use of patterns and templates has facilitated rapid and uniform fabrication and has simplified the training of bamboo workers. Several designs have been established and production methods worked out whereby a number of articles, such as highly serviceable chairs, benches, couches, folding tables, and stools are being manufactured.

With increased shop production, considerably more attention has been diverted to the cutting, trimming, and subsequent handling of the bamboo intended for use in the construction of furniture and other articles. Various methods of field curing have been tried, and the most satisfactory to date has been that in use at the station for several years, namely, to cut the culms and leave them upright in the field with the branches and leaves attached for a few weeks. After trimming, an additional period of several weeks in ventilated dry storage has been found necessary for complete conditioning.

Miscellaneous: Howard T. Love.

Experiments conducted at this station during 1942 indicated that bamboo, like some other woods, can be plasticized by treating with urea solution. The treatment of thin narrow strips of bamboo, used for the preparation of woven canelike seats and backs of chairs, by soaking in 10-percent urea solution for 24 hours, permitted bending through a sharp angle of 90° without fracturing. The strips were most pliable when heated in the urea solution and bent while hot.

Ribless leaves of the royal palm (*Roystonea regia* (H. B. K.) Cook) are used extensively in Puerto Rico for weaving chair seats and backs of bamboo and other furniture, the cured leaves ordinarily being moistened with water and twisted by hand into cords. On becoming dry some of the fibers of the cords tend to fracture. To overcome this tendency an experiment was carried out in cooperation with the Puerto Rico Development Co., in which dried palm leaves were soaked in a

[7] The diameter of each culm was measured at the center of the internode nearest to the 5-foot elevation above ground. Wherever a node was located at this height, the caliper of the internode next below was recorded.

10-percent urea solution for 12 to 48 hours. When air-dried or when placed in the shade in a humid atmosphere the leaves thus treated remained more pliable than similar untreated leaves under the same conditions. When oven-dried or dried in the sun they became as brittle as the untreated leaves. There were no apparent differences in these respects among lots soaked 12 hours and 48 hours at room temperature, treated with hot urea solution, and untreated. The conclusion reached was that a single urea treatment at least temporarily made the leaves more pliable. The effect of the urea appeared to be more that of maintaining a higher moisture content in the leaves than acting as a plasticizer.

ESSENTIAL OILS

Processing studies: Noemí G. Arrillaga.

During the past year a few small plantings of lemon grass (*Cymbopogon citratus* (DC.) Stapf.) have been made by various growers throughout the island, and the first lemon-grass oil was distilled commercially.

Experiments with citronella grass (*Cymbopogon nardus* (L.) Rendle) on drying and haying have shown that about three-fourths of the oil was lost during drying and storage of the grass prior to distillation.

An experiment to determine the value of exhausted lemon and citronella grass as fuel indicated that the dried grass had a fuel efficiency about equal to that of wood and would be worth considering as a possible source of part of the fuel required for distillation.

Experiments in the distillation of essential oil from vetiver (*Vetiveria zizanioides* Stapf.), locally known as "pachulí," showed that the rate of entrainment of oil in units of oil distilled per unit of water was 5 times greater during the first 3 hours and 2 times greater during the fourth and fifth hours from chopped roots than from whole or ground roots. This was ascribed to the fact that the oil was not readily available in the whole roots and that channeling occurred in the finely ground roots. Soaking the roots in water prior to distillation did not increase the rate of entrainment of oil.

In a comparison of flower absolutes from *Coffea arabica* L. and *C. robusta* Linden it was found that the former species yielded a product having a somewhat stronger and more agreeable aroma. The yields of absolute, as determined by Soxhlet extraction with petroleum ether, were 0.65 and 0.62 percent fresh basis for the first and second flowerings of *C. arabica* and 0.65 and 0.66 for corresponding flowerings of *C. robusta.*

Forty pounds of *Coffea arabica* flowers and 97 pounds of *C. robusta* flowers were extracted in a modified percolator with petroleum ether purified by distillation from lard. The absolute, purified in the usual manner, amounted to 0.49 percent for *C. arabica* and 0.52 for *C. robusta.* Thus the extraction with this apparatus was only approximately 79 percent as efficient as the Soxhlet extraction.

The use of unsaturated compounds, such as ethylene, acetylene, and carbon monoxide for inducing the abscission of leaves, flowers, and buds of various plants has been reported by various writers. Such a treatment would be advantageous for harvesting essential-oil-bearing flowers and leaves if it could be carried out on a practicable basis.

Applied to freshly cut, flowering ilang-ilang branches under bell jars at a concentration of 1:10,000, ethylene induced the fall of ripe petals, acetylene appeared to cause some ripening but no fall, and carbon monoxide resulted only in a darkening of the flowers. However, in field tests, spraying saturated aqueous solutions of ethylene, with and without a spreader, on ilang-ilang trees failed to produce any effect. Some method of enclosing the trees to decrease air circulation would probably be necessary before satisfactory results could be obtained in the open.

Miscellaneous: Merriam A. Jones.

Because of the shortage of tanning material on the island, a brief investigation was made of the tannins obtainable from the water left in the stillpot after distillation of the oil from the leaves of the bay tree (*Pimenta racemosa* (Mill.) Moore). Various chemical tests were made in order to characterize these tannins and to find what others they resembled. Iron tests showed that the substance has iron-bluing as well as iron-greening properties. Phloroglucinol was indicated along with a phlobaphene body, aromadendrin, and kinoyellow. These tests and others indicated that the tannins resemble in some respects those of some species of *Eucalyptus* and *Angophora*, as well as the tannins of cube gambier, acacia catechu, and cutch (*3*, *13*).

On a dry basis bay leaves were found to contain 32 percent hot-water-soluble matter of which about three-fourths could be obtained in one extraction if provision were made for good drainage of the leaves. Comminution of leaves with water at room temperature also gave satisfactory extracts. In order to concentrate the extracts several methods could be used. Although absorption on solids and precipitation by lead salts or sulfuric acid were found to be unsatisfactory, solvent extraction appeared feasible. However, simple evaporation, either under vacuum or at atmospheric pressure, was considered best. Under the vacuum of a water jet, extracts boiled as low as 37° C. Using such vacuum and solar energy to furnish the latent heat of vaporization of water, an economical process of concentration could be devised. On the basis of the island production of about 35,000 pounds of bay oil per year, about 100,000 pounds of tannin are available, and about 75,000 pounds of this could be recovered by evaporation.

SPICE CROPS

Production studies: Carmelo Alemar, Jr.

Excellent production was obtained in experimental work on the growing of the Chinese variety of ginger (*Zingiber officinale* Rosc.). Two readily available materials, well-decomposed sugarcane filterpress cake, or cachaza, and well-decomposed leaves of various tree species, were tested as mulches for the growing of ginger on clay loam soil containing a considerable amount of sand. On an acre basis, the yields were 37,318 pounds and 32,136 pounds for the respective mulch treatments and 16,727 pounds for the check. Statistical analysis of the data showed that the differences among yields were highly significant. There is every indication that the growing of Chinese ginger in Puerto Rico would be highly profitable. The multiplication of planting material of this variety, which is superior to the native variety now commonly grown, is being extended as rapidly as possible.

VANILLA

Production studies: Ernesto Hernández Medina and Jacinto Rivera Pérez.

During the year many of the vanilla experiments made to test the effect of various soil alterations reached the production stage and flowered for the first time. At the end of approximately 2 years, the average stem growth per living plant was 12.6 feet in those treatments in which mulch was mixed with soft limestone, 11.5 with mulch over the limestone, and 10.5 with mulch alone. The percentage of healthy plants was 96.3, 86.3, and 91.3, respectively, while the pH of the respective mulches was 7.27, 7.23, and 6.93. The number of flowers pollinated this year was 50, 8, and 32 for the respective treatments. Approximately one-half of the flowers produced were pollinated in each treatment, since previous experiments have shown this to be optimum for the best production of beans and the health of the plants. In the treatment of mulch mixed with soft limestone, the number of flowers produced per plot was somewhat lower than when mulch alone was used, yet the total number of flowers pollinated was greater than in the other two treatments combined.

In another experiment with 56 plants in 7 replicated plots per treatment, 45 flowers were pollinated in all of the plots on mulch mixed with gravel, 38 flowers in 1 plot on mulch on soft limestone, 20 in 3 plots of mulch on gravel, and 9 in 1 plot on mulch mixed with soft limestone.

In another experiment in which well-decomposed cachaza was used as a mulch, there was an increase in the average stem growth per plant but the number of healthy plants was reduced, and likewise the number of flowers produced was considerably less than when leaf mulch was used alone.

The results obtained thus far in the soil-alteration experiments indicate generally that the addition of soft limestone to the mulch, providing better aeration and drainage, encourages superior early vegetative growth, higher survival of the plants, and heavier flowering the first producing year.

In connection with nutrient studies to be undertaken on the growth of vanilla, an orientation experiment was made to find a basic nutrient solution suitable for the growing of vanilla in sand culture. Four solutions, reported to be suitable for the growing of orchids, were tried: Hoagland and Arnon T. C. solution, one-half strength; Wagner and Poesch W. P. solution, one-half and three-fourths strengths; and Knudson solution B.

In root development and vegetative growth the W. P. solutions surpassed the other two. The weight of vegetative growth produced was significantly higher than any other with both concentrations of the W. P. solution. Data on leaf surface also gave a similar trend. From the results obtained, it was apparent that vanilla could be grown satisfactorily in sand culture using either a one-half or three-fourths concentration of the Wagner and Poesch solution, with the results of this experiment slightly favoring the stronger concentration.

Observations on vanilla planted on three different support trees, cashew (*Anacardium occidentale* L.), bucare (*Erythrina berteroana* Urban), and bauhinia (*Bauhinia reticulata* DC.), at Mayaguez

showed a decided superiority in growth in those vines growing on cashew. Plantings were made on bench terraces constructed on steep land having a western exposure and without wind protection or additional shade. The vanilla plants on the cashew were planted in May 1941, at which time the support trees were approximately 2 years old and about 4 to 5 feet in height and had from 3 to 4 feet lateral spread of dense foliage. After approximately 2 years' growth 39 of 40 of the vanilla plants were alive and flourishing, and 17 had bloomed. The cashew, which does not drop its leaves, apparently provides, without additional shade protection, a quantity of light that is highly favorable to the growth of vanilla.

On adjacent terraces where vanilla of the same age or older was planted on bucare and bauhinia, the plantings were in poor condition. Over half of the vines planted on these support trees have died, and the remaining living plants have made poor vegetative growth. In spite of the fact that some of these vines are actually older than those growing on cashew, only slight flowering of the vanilla has taken place on bucare and none on bauhinia. Further experimentation with cashew as a support tree is planned, but, on the results observed thus far, it can be recommended as a support tree for vanilla, particularly on open hillsides on the western end of the island.

Under commercial conditions vanilla flowers must be hand-pollinated to produce beans, for if pollination is left to natural agencies the crop of beans will be so small as to be unprofitable. Artificial or hand pollination requires considerable skill and time on the part of the operator. An experiment was started to investigate the possibility of bringing about fruit formation without pollination, i. e. parthenocarpically, by the use of such growth-promoting chemicals as have been similarly used with success on other plants.

Following the suggestions of P. W. Zimmerman, Boyce Thompson Institute for Plant Research, visiting scientist at the Institute of Tropical Agriculture, Mayaguez, and W. C. Cooper, Office of Foreign Agricultural Relations, cooperating with the station, solutions of five such chemicals were selected for preliminary trial. These solutions were prepared with distilled water to concentrations ranging from 10 to 3,000 milligrams per liter, depending on the chemical, and sprayed by means of a small atomizer onto the corolla of vanilla flowers in various stages of development. When applied to clusters of very immature flower buds, e. g., buds that would open in about a month, the chemicals caused the individual buds to rot and abscise. On flower buds due to open in 4 or 5 days, on open flowers, and on spent flowers the chemicals did not produce these effects, but caused the corolla to adhere to the ovary from 3 days to several months longer than is normal with unpollinated flowers. In addition, the chemicals distorted the sepals and petals of the unopened flowers to such an extent as to change radically the form of the flower, and such flowers did not open completely. Wherever beans set as a result of chemical treatment, they developed much more slowly than those resulting from hand-pollination.

Of the chemicals used, 2,4-dichlorophenoxyacetic acid at 50 milligrams per liter has thus far given the most promising results; more than 75 percent of the flowers sprayed with this material produced fruit, and few of the developing beans have abscised. Of the beans

examined none was found to contain seeds. While of a preliminary nature, these results offer some hope of finding a rapid method of pollinating vanilla flowers; however, considerable work remains to be done and the type and quality of the resultant beans must be determined before any evaluation of the method can be made.

Processing studies: Francisca E. Arana.

An experiment was conducted to compare the effects of seven well-known vanilla-bean killing procedures followed immediately by each of two sweating methods, sun and oven sweating. After sweating, the beans were dried and then conditioned in groups to contain approximately 26, 32, and 52 percent moisture, respectively. Data were taken on the tendency to mold and split, loss in weight during the conditioning period, phenol content, and physical qualities of the resulting cured beans.

The more effective killing treatments, such as hot water, sun, freezing, and ethylene gas, resulted in no appreciable amount of molding when the beans contained less than 26.5 percent moisture. However, an average of 3 percent of the vanilla beans killed by the ethylene treatment was found to be moldy when cured to a moisture content of 33.6 to 54.3 percent. Of the sun-cured beans with a 30.9-percent moisture content 4 percent were found to be moldy, while an average of 7 percent of those similarly cured to 53-percent moisture molded. On the other hand, in beans killed with hot water and by freezing no appreciable mold occurred, even when cured to a moisture content of 50.5 percent. The ethylene gas treatment has been considered to be a maturing rather than an actual killing treatment. Likewise, sun killing is a slow method as compared with dipping in hot water or subjecting to freezing temperatures. It was, therefore, concluded that in those procedures in which the beans were not thoroughly and quickly killed there was a strong tendency to develop mold, especially in cured beans having a high moisture content.

Least splitting occurred in the beans that were killed by the pin-scratching, oven, and hot-water procedures and cured to contain about 35 percent moisture. Oven sweating resulted in less mold and less splitting than sun sweating following all killing procedure used except oven killing.

When the beans were examined for loss in weight during the conditioning period, the observations made during previous years were confirmed. Beans which lost the greatest amount of moisture during the curing process lost the least during the 3-month conditioning period. Beans cured to approximately 30 percent of their original weight lost one-half as much moisture during the conditioning period as those cured to approximately 55 percent.

Within groups the phenol content did not vary with moisture content. As to appearance, the beans with the lowest moisture content were generally darker brown in color than all the others except the frozen beans, which were characterized by a uniform reddish-brown color. The aroma of these dry beans was generally more well-developed and suave, the vanillin crystallization was greater, and the flexibility less. However, the frozen beans were characterized by considerable flexibility, even when their moisture content was low. The beans with moisture content ranging from 49.9 to 54.3 percent had a slightly fermented aroma and lacked suavity.

Vanilla beans harvested with the blossom ends yellow result in a cured product superior to that from beans harvested green. However, beans at the blossom-end-yellow stage have a tendency to split on the vine or in curing, and this is objectionable because split beans are penalized on the market. The splitting generally occurs along two well-developed abscission layers. In an attempt to prevent this splitting so that the beans could be harvested with the blossom ends yellow, an experiment was conducted in which growth-regulating substances were employed such as those used to prevent the preharvest drop of apples. Concentrations of 1-naphthaleneacetic acid ranging from 50 to 1,000 milligrams per liter of water were tried, with sodium alginate and sodium isopropyl naphthalenesulfonic acid as spreaders. The beans were treated on the vines by dipping in the solution at 2-week intervals over a period of 6 to 10 weeks before harvest. When about one-third yellow, the beans were harvested and killed by dipping in hot water and then cured. Beans on the same and on different vines served as controls. Under the conditions of the experiment, no significant control of splitting was obtained. Treatment of harvested beans with the vapors of ethyl 1-naphthaleneacetate under a bell jar yielded similar results.

SOIL CONSERVATION

Soil and moisture conservation: Joel W. Elliott and Miguel A. Maymon, Soil Conservation Service, U. S. Department of Agriculture.

Work carried on by the Soil Conservation Service at the station has shown that food production in Puerto Rico may be increased by bench-terracing the thousands of acres of good soil now unproductive or uncultivated on hillsides having a slope of 20 to 50 percent. Ten normal crop plowings against stiff-stemmed grass-barrier lines having a 0.25- to 1.0-percent grade broke up the original land slope of a Catalina clay hillside into a kind of stairway resembling a series of bench terraces and moved about 90 percent of the soil necessary to convert this stairway into bench terraces of an acceptable cross section. A barrier plant was not absolutely necessary after the first 7 or 8 plowings; however, several grasses each quickly formed dense barriers with few gaps when planted 6 inches apart in 2 rows. The most desirable barrier grasses, in the order named, were elephant grass, Guatemala grass, and sugarcane.

Centipede grass was the best bank-soil binder of 4 soilage grasses observed over a 5-year period. Banks were utilized by planting cucumbers and native squash 1 foot back from the terrace rim and later training the vines over the rims and down the undisturbed, grassed banks. Furrows parallel to the terrace rim decreased bank washing and were more economically formed and cultivated than furrows at an angle to the flow line or parallel to the flow line along the terrace heel. Depending on the time of planting, tomatoes, cabcage, and corn have grown well in these furrows. Turning under green-manure crops in the innermost section of the terrace bench improved the yield of corn and sweetpotatoes.

For the protection of terrace outlet channels strip sodding failed, as overfalls developed below each strip; sprigging proved good when

waterflow could be deflected to other channels until the grass took root, but solid sodding was preferable where the deflection was not possible. Sour paspalum grass was found to be more adaptable to clay soils and to a wider range of light conditions than Bermuda grass.

In the vegetative control of erosion on the several thousand acres of steep hillsides in Puerto Rico now being used as pasture or soilage areas, a 2-year native-grass-pasture fertilizer test on Cialitos clay proved that nitrogen in combination with any one or all of the other fertilizer constituents excepting potash alone, or with lime alone, produced significant increases in forage production.

In a grazing test of trailing indigo, a test area of the legume was completely covered and protected from erosion with a new growth of vines within 3 weeks after having been pastured for 10 days during the rainy season to a mat of woody stems. Reports from local cooperators indicated that this legume may be used as a pasture plant in erosion control without visible toxicity symptoms appearing in the unconfined farm livestock usually found in Puerto Rico.

Studies made on Catalina clay showed that bare uncultivated subsoil and bare uncultivated topsoil permitted a runoff of 47.04 and 46.53 percent and the erosion of 8,346 and 6,244 pounds of soil, respectively, per acre per inch of rainfall averaging 0.75 inch per hour in intensity. On the other hand, when the soil was planted to sour paspalum grass, for example, the runoff was only 5.13 percent and the erosion only 6 pounds under the same rainfall conditions.

The rate of runoff from plots of bare soil and of certain grasses was positively influenced by average rate of rainfall, quantity of rainfall per rain, moisture in the soil before rains, and height of vegetation. On erosion in the grass plots these factors had no effect, but their influence on bare soil was positive and of high significance.

COFFEE

Variety trials: In cooperation with Jaime Guiscafré and Luis A. Gomez, University of Puerto Rico Agricultural Experiment Station.

For the ninth consecutive year the Columnaris variety of *Coffea arabica* out-yielded the West Indian variety. The average yields for 9 years are now 1,215 pounds and 654 pounds per acre, respectively. A surprising difference occurred during the past year; the yield for the Columnaris dropped considerably and was the lowest since 1934, whereas the West Indian variety had the highest yield since 1939, the averages for the 1942 crop being 931 pounds and 750 pounds per acre, respectively.

PUBLICATIONS

Editorial: Harold K. Plank.

As during the previous fiscal year, the development of the various projects being carried out at the station during 1943 was summarized in quarterly reports. These reports totaled 75 mimeographed pages. Sixty-nine copies of each issue were circulated among workers in other offices of the Department or sent on request to individuals professionally interested in the current work of the station.

The annual report for the fiscal year 1941, reduced to 24 pages because of war conditions, was issued in December 1942 and sent on request or exchange to 1,135 institutions and individuals throughout the world. Of these, 743 requests were from the continental United States and possessions and 392 from 42 foreign countries. The annual report for the fiscal year 1942, consisting of 29 pages, was issued in June and distributed approximately on the same basis.

The following specialized articles concerning work carried out at the station were published during the year through sources outside the Department:

ARRILLAGA, NOEMÍ G. New essential oils from Puerto Rico. Amer. Perfumer and Essential Oil Rev. 45 (1) : 27–29, illus. 1943.

ARRILLAGA, NOEMÍ G. Absolute from coffee flowers. Amer. Pharm. Assoc. Jour., Sci. Ed. 32 (3) : 77–79, illus. 1943.

ARRILLAGA, NOEMÍ G., and JONES, MERRIAM A. Use of salt in distilling bay leaves, [III]. Amer. Perfumer and Essential Oil Rev. 44 (9) : 29–31. 1942.

HORN, CLAUD L. The frequency of polyembryony in 20 varieties of mango. Amer. Soc. Hort. Sci. Proc. 42 : 318–320, illus. 1943.

HORN, CLAUD L. and ARROYO, ARMANDO. New bamboo revetment construction. Mil. Engin. 35 (212) : 284–286, illus. 1943.

HORN, CLAUD L., and NATAL COLÓN, JOSÉ E. Acid scarification of the seed of two Cuban fiber plants. Amer. Soc. Agron. Jour. 34 : 1137–1138, illus. 1942.

JONES, MERRIAM A., and ARRILLAGA, NOEMÍ G. Use of salt in distilling bay leaves. [II]. Amer. Perfumer and Essential Oil Rev. 44 (8) : 25–28, illus. 1942.

JONES, MERRIAM A., and ARRILLAGA, NOEMÍ G. Use of salt in distilling bay leaves. [IV]. Amer. Perfumer and Essential Oil Rev. 44 (10) : 25–29, illus. 1942.

LITERATURE CITED

1. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.
   1940. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS . . . Ed. 5, 757 pp., illus. Washington, D. C.
2. BARRETT, O. W.
   1928. THE TROPICAL CROPS. 445 pp., illus. New York.
3. DREAPER, W. P.
   1911. TANNINS. *In* Allen, A. H., Allen's commercial organic analysis. Ed. 4, v. 5, pp. 1–104. Philadelphia.
4. EATON, F. M.
   1936. AUTOMATICALLY OPERATED SAND-CULTURE EQUIPMENT. Jour. Agr. Res. 53 : 433–444, illus.
5. GOODHUE, L. D.
   1936. AN IMPROVEMENT ON THE GROSS AND SMITH COLORIMETRIC METHOD FOR THE DETERMINATION OF ROTENONE AND DEGUELIN. Assoc. Off. Agr. Chem. Jour. 19 : 118–120.
6. ———and HALLER, H. L.
   1939. A METHOD FOR DETERMINING DEGUELIN IN DERRIS AND CUBE. Indus. and Engin. Chem., Analyt. Ed. 11 : 640–642.
7. ——— and HALLER, H. L.
   1940. DETECTION AND ESTIMATION OF DIHYDROROTENONE IN THE HYDROGENATION PRODUCTS OF ROTENONE. Indus. and Engin. Chem., Analyt. Ed. 12 : 652–654, illus.
8. HOAGLAND, D. R., and ARNON, D. I.
   1938. THE WATER-CULTURE METHOD FOR GROWING PLANTS WITHOUT SOIL. Calif. Agr. Expt. Sta. Cir. 347, 39 pp., illus.
9. JONES, H. A., CAMPBELL, F. L., and SULLIVAN, W. N.
   1935. RELATIONS BETWEEN CHEMICAL COMPOSITION AND INSECTICIDAL EFFECTIVENESS OF ROTENONE-BEARING PLANTS. Jour. Econ. Ent. 28 : 285–292.
10. LOVE, H .T.
    1943. AVOCADO OIL STUDIES. Puerto Rico (Mayaguez) Agr. Expt. Sta. Rpt. 1942 : 18–19.

11. MITCHELL, J. W., and STUART, N. W.
1939. GROWTH AND METABOLISM OF BEAN CUTTINGS SUBSEQUENT TO ROOTING WITH INDOLEACETIC ACID. Bot. Gaz. 100: 627–650, illus.
12. NEW YORK ACADEMY OF SCIENCES.
1925. SCIENTIFIC SURVEY OF PUERTO RICO AND THE VIRGIN ISLANDS. v. 6, 663 pp. New York.
13. NIERENSTEIN, M.
1927. TANNINS. *In* Allen, A. H., Allen's commercial organic analysis. Ed. 5, v. 5, pp. 1–204. Philadelphia.
14. STUART, N. W.
1938. NITROGEN AND CARBOHYDRATE METABOLISM OF KIDNEY BEAN CUTTINGS AS AFFECTED BY TREATMENT WITH INDOLEACETIC ACID. Bot. Gaz. 100: 298–311, illus.

○